Fentanyl Pharmacokinetics

the role of clinical factors in patients with cancer using transdermal or sublingual fentanyl

Evelien Kuip

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Fentanyl farmacokinetiek

– de rol van klinische factoren bij patiënten met kanker die transdermaal of sublinguaal fentanyl gebruiken –

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General introduction and outline of the thesis

General introduction

Pain in patients with cancer

Pain is a common problem in patients with cancer which compromises quality of life. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (1). Pain may be caused by cancer --or its treatment-- either in curative or palliative setting. A recent meta-analysis showed high pooled prevalence rates of cancer-related pain; 39% in patients treated with curative intention, 55% during anti-cancer treatment, and 66% in non-curative settings (2).

Cancer related pain can be distinguished in continuous (or baseline) pain and breakthrough pain. Background or baseline pain is defined as 'constant or continuous pain of long duration' of at least 12 hours per day (3). Besides background pain, 40 - 80% of the patients with cancer related pain suffer from breakthrough pain (4). Breakthrough pain is defined as 'a transient exacerbation of pain that occurs either spontaneously, or in relation to a specific predictable or unpredictable trigger, despite relatively stable and adequately controlled background pain' (5). Of patients with breakthrough pain, 39% reports spontaneous-type pain, 44% incident-type pain, and 17% a combination of these pains (6).

Pain has a large impact on the quality of life. It limits daily physical activities and has (therefore) also an impact on psychological and social aspects of daily life (7-10). Generally, pain management needs a multimodality approach with attention for psychological, social, and spiritual components, besides the pure physical component. This approach follows the concept of 'total pain', first mentioned by Dame Cicely Saunders, the founder of palliative care, and currently widely adopted, in particular in palliative care (11). Pain is often measured by the use of the Numerical Rating Scale (NRS) (12). Patients are asked to give an indication of their pain, 0 represents no pain at all, and 10 represents the worst pain imaginable. In daily clinical practice, pain scores of $> 4 - \leq 6$ are labelled as moderate pain and $\geq 7 - 10$ as severe pain, although there is still need for more evidence on the optimal cut points (13, 14). A decrease of at least two points on the NRS is considered clinically relevant (12).

As a starting point for the pharmacological treatment of pain the World Health Organization (WHO) initiated the analgesic pain ladder (15). Treatment starts with non-opioids +/- an adjuvant (step 1), when pain persists or increases weak opioids can be added (step 2) and when step 2 is insufficient strong-opioids are used (step 3). However, for patients with cancer step 1 is directly followed by step 3 according to several guidelines (16-19). Application of this approach is successful in approximately 80-90% of the patients (16, 18, 20, 21). Although the WHO pain ladder advises to use opioids it does not specify *which* opioid to start with. There are several opioids available in the Netherlands like morphine, fentanyl, oxycodone, hydromorphone and

methadone. All opioids have been proven to be effective in cancer related pain, but the response of individual patients may vary largely for different types of opioids (18, 22). Unfortunately, physicians cannot predict the response on a specific opioid for the individual patient. So after the start of an opioid patients need to be monitored to evaluate the effects and the side effects of the pain treatment (23).

Fentanyl

Fentanyl is one of the strongest acting opioids with an 80-100 times larger affinity for the opioid receptor compared to morphine. One of the pharmacological characteristics of fentanyl is that it is highly lipophilic and has a strong binding capacity to plasma proteins (24, 25). Fentanyl is considered to be metabolized in the liver by cytochrome P450 iso-enzyme 3A4 (CYP3A4) resulting in the inactive metabolite norfentanyl (26, 27). Besides norfentanyl less than 1% of the metabolites consists of the other inactive metabolites hydroxyfentanyl, hydroxynorfentanyl and despropionylfentanyl. In the end almost 10% of the intact fentanyl molecule and all of the metabolites are excreted by the kidneys (28, 29).

Because of its lipophilicity, fentanyl is suitable for both transcutaneous and transmucosal administration routes. Already since the early 1990's, fentanyl is used in a transdermal patch for the treatment of continuous cancer-related pain (30). Fentanyl patches which are currently on the market, consist of a silicone-based adhesive to attach the patch to the skin and a drug reservoir that contains fentanyl and an ethanol gelled hydroxyethyl cellulose (26, 31, 32). Fentanyl is absorbed by the skin and enters via the microcirculation of the skin the systemic circulation and is subsequently rapidly distributed. Over the last years several immediate release fentanyl preparations, so called rapid onset opioids (ROO's), became available for the transmucosal administration routes (24, 33, 34). These products are used to treat breakthrough pain. Buccal tablets have to be placed between upper gum and cheek above a molar tooth, whereas sublingual tablets are aimed to be used under the tongue. The fentanyl in these products is absorbed by the oral mucosa and afterwards enters the systemic circulation (35). Fentanyl in nasal sprays is rapidly absorbed by the highly vascularized nasal mucosa (36).

Pharmacokinetics

The basis of modern pharmacotherapy is pharmacodynamic and pharmacokinetic information. Pharmacodynamics (PD) describe the effect that drugs have on the body; the effects and the side effects or toxicity. In case of fentanyl, analgesic, or pain killing, effects may occur together with the side effects like nausea, obstipation, sleepiness, respiratory depression (37). Both beneficial and side effects are related to the exposure to fentanyl and is determined by pharmacokinetic aspects. Pharmacokinetics (PK) describe the process of the body handling the drug (e.g. absorption, distribution, metabolism and elimination). The pharmacokinetic exposure to a drug is typically described using the maximum plasma concentration (C_{max}) and, the area under the curve (AUC), representing the drug exposure over time.

Fentanyl is widely known for its wide intra- and inter-patient variability in pharmacokinetics and the cause of this variety is not completely understood (38-40). In a fentanyl patch, fentanyl is thought to be released from the patch at a more or less constant rate for 72 hours (23, 33), although a recent pharmacokinetic study in patients using transdermal fentanyl showed plasma concentration fluctuations during transdermal treatment which closer fitted first-order than zero-order kinetics (33). This suggests a more concentration-dependant absorption process in which the absorption rate declines with a decreasing fentanyl concentration inside the patch. Although the reason for PK variation is unclear, the amount of subcutaneous fat might influence the absorption rate or bioavailability of fentanyl. Unfortunately there is only limited research on the influence of factors related to the thickness of the subcutaneous fatty layer like body mass index (BMI) or the localization of the patch on the body (41, 42). Since the amount of subcutaneous fat may vary substantially with progressing cancer disease and between different localizations of the body, this is an highly unmet need. Another factor that typically changes during different phases of cancer treatment is the use of comedication. Patients with cancer use various anti-cancer drugs and specific medications to treat side effects of the used anti-cancer therapy or other symptoms, complications and comorbidity e.g. nausea or infectious diseases. These concurrently used medications are quite often metabolized by CYP3A4, just like fentanyl. The combination of fentanyl and other, CYP3A4 metabolized medication might therefore influence plasma fentanyl levels, risking higher or lower levels of fentanyl than expected.

Additionally cigarette smoking is a factor that indirectly might influence CYP3A4 metabolism by induction of CYP iso-enzymes caused by the polycyclic aromatic hydrocarbons in cigarette smoke and by this mechanism might influence fentanyl PK (43, 44). Although smoking is unquestionably a habit that changes during different phases of the cancer disease, to our knowledge, the influence on fentanyl PK has not been studied.

For the oromucosal fentanyl products a relatively low bioavailability has been found (50-70%) due to partial loss of fentanyl because of first pass metabolism caused by gastrointestinal absorption rather than direct uptake by the mucosa (24, 35). The nasal products have a higher bioavailability because of the small volume of fentanyl that is directly absorbed by the nasal mucosa whereby hardly any loss occurs by swallowing fentanyl (45). Besides variation in bioavailability, T_{max} varies widely, especially for the oromucosal fentanyl products. For these oromucosal fentanyl products T_{max} varies from 30-240 minutes, while for nasal fentanyl products T_{max} is much shorter (11-20 min) and with a smaller interindividual variability (35).

Although this variation is observed repeatedly, literature about factors associated with this variation is sparse. Regrettably most PK research on transmucocal fentanyl has been done in healthy volunteers and results might not be the same in patients with cancer related pain, especially not for patients suffering from mucosal oedema, mucositis or xerostomia. This lack of PK studies complicates decision making for the optimal pain management in this vulnerable patient population during the cancer disease trajectory.

Aims of this thesis

Finding the right dose of fentanyl for the individual patient is achieved by titration and often refers to a delicate balance between sufficient pain relief and the manifestation of side effects. Subsequently more knowledge of factors that potentially influence fentanyl PK might lead to earlier adaptations in fentanyl dose in individual patients during the cancer disease trajectory.

We started this thesis with a review of the literature on factors that had been studied in relation with fentanyl PK. The lack of research performed in patients with cancer to unravel factors that influence fentanyl PK was the main reason to perform the prospective research in this thesis. We focused our research on common patient characteristics prone to change during different phases of the disease in patients with cancer using trandermal or sublingual fentanyl and studied whether these factors affected fentanyl PK.

The research questions addressed in this thesis are as follows:

1) To report which factors were studied in relation to fentanyl pharmacokinetics and might contribute to the variation in fentanyl pharmacokinetics.
2) To study the influence of common patient characteristics, BMI and smoking, on fentanyl exposure in patients with cancer.
3) To study the effect of a regularly used CYP3A4 inhibitor, aprepitant, on fentanyl exposure in patients with cancer.
4) To investigate the role of the localization of the fentanyl patch onfentanyl exposure in patients with cancer.
5) To study the effect of mucositis on the exposure of sublingually delivered fentanyl in patients with head and neck cancer treated with chemo- or bioradiotherapy.
6) To explore the effect of xerostomia (dry mouth) on the exposure of sublingually delivered fentanyl in patients with head and neck cancer after treatment with chemo- or bioradiotherapy.

Outline of this thesis

In **chapter 2**, a systemic review of investigated factors that potentially may influence fentanyl pharmacokinetics is reported. Factors were split in in four groups: pharmacokinetic drug-drug interactions, environmental factors, patient related factors and pharmacogenetics.
In **chapter 3**, we describe methods for bioanalysis of opioid concentrations in plasma of patients with cancer. The used methods are validated for analysis of fentanyl, norfentanyl, morphine, hydromorphone and the metabolites morphine-3ß-glucuronide and morphine-6ß-glucuronide.
In **chapter 4**, we present a study on the influence of the common patient characteristics, variation in BMI and smoking yes or no, on the exposure of transdermal fentanyl in patients treated for cancer related pain. We aimed to partly explain the wide differences in fentanyl pharmacokinetics between individual patients.
In **chapter 5**, we present results of two separate studies investigating the effect of the localization of the fentanyl patch, upper arm versus thorax, and the use of the moderate CYP3A4 inhibitor aprepitant, on the exposure of transdermal fentanyl. These factors were not earlier studied in patients with cancer and might explain (a part of) the variety in fentanyl pharmacokinetics.
In **chapter 6**, the results of a prospective study in patients with head and neck cancer treated with chemo- or bioradiotherapy is reported. We studied the influence of mucositis on the exposure to sublingually delivered fentanyl using an intra-patient comparison. Besides we describe the effect of xerostomia on the exposure to sublingually delivered fentanyl in these patients six weeks after the end of chemo- or bioradiotherapy .
In **chapter 7**, a summary of the studies described in this thesis, a discussion on the methods and findings and recommendations for further research are given.

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2

A review of factors explaining variability in fentanyl pharmacokinetics; focus on implications for cancer patients

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Abstract

Fentanyl is a strong opioid that is available for various administration routes, and which is widely used to treat cancer-related pain. Many factors influence the fentanyl pharmacokinetics leading to a wide inter- and intra-patient variability. This systematic review summarizes multiple studied factors that potentially influence fentanyl pharmacokinetics with a focus on implications for cancer patients. The use of CYP3A4 inhibitors and inducers, impaired liver function, and heating of the patch potentially influence fentanyl pharmacokinetics in a clinically relevant way. In elderly patients, current data suggest that we should carefully dose fentanyl due to alterations in absorption and metabolism. The influence of BMI and gender on fentanyl pharmacokinetics is questionable; most probably due to a large heterogeneity in the published studies. Pharmacogenetics, e.g. the *CYP3A5*3* gene polymorphism, may influence fentanyl pharmacokinetics as well, although further study is warranted. Several other factors have been studied but did not show significant and clinically relevant effects on fentanyl pharmacokinetics. Unfortunately, most of the published papers that studied factors influencing fentanyl pharmacokinetics describe healthy volunteers instead of cancer patients. Results from the studies in volunteers may not be simply extrapolated to cancer patients because of multiple confounding factors. To handle fentanyl treatment in a population of cancer patients, it is essential that physicians recognize factors that influence fentanyl pharmacokinetics, thereby preventing potential side-effects and increase its efficacy.

Introduction

Pain is a common and relevant problem in cancer patients (1). Chronic pain occurs in about 30-50% of cancer patients undergoing curatively aimed treatment and in 70-90% of patient with advanced disease (2). Breakthrough pain is found in 64-90% of cancer patients with chronic pain (3). It is defined as a '*transitory exacerbation of pain experienced by the patient who has relatively stable and adequately controlled baseline pain*' (4, 5). Moderate-severe cancer pain is commonly treated with opioids, of which fentanyl is one of the most widely used, especially because it is available for various easy to use administration routes. Since the 1990s the fentanyl transdermal patch can be used to treat chronic pain (6). Nowadays, there are also several rapid onset products available for the treatment of breakthrough pain: products for transmucosal, buccal, sublingual, and even intranasal administration (7).

Fentanyl is a strong opioid (approximately 75 – 100 times more potent than morphine), highly lipophilic and binds strongly to plasma proteins (8, 9). Its volume of distribution is large (3.5-8 L/kg) and its clearance relatively high (30-72 L/h) (9). Fentanyl is thought to be predominantly metabolized in the liver by cytochrome P450 iso-enzyme 3A4 (CYP3A4)-mediated N-dealkylation resulting in the inactive metabolite norfentanyl. Less than 1% is metabolized by alkyl hydroxylation, N-dealkylation or amide hydrolysis to the inactive compounds hydroxyfentanyl, hydroxynorfentanyl and despropionylfentanyl. The inactive metabolites, and approximately 10% of the intact molecule, are mainly excreted by the kidneys (10-12). (**Figure 1**). Although this is the most accepted explanation of fentanyl metabolism, a recent study showed that the CYP3A4-mediated N-dealkylation step may not be as important as always thought. Unknown metabolic routes may be responsible for a significant part of fentanyl metabolism (13).

When fentanyl is given as an intravenous bolus, fentanyl is rapidly distributed from plasma into highly vascularized compartments. After uptake in the systemic circulation, redistribution to muscle and fat tissue occurs. Elimination half time is highly variable in various studies (219 min – 853 min), particularly due to this redistribution (14-17). Fentanyl used in a transdermal patch is absorbed first by the skin, then taken up into the cutaneous microcirculation, followed by entering the systemic circulation. The rapid-onset fentanyl products are absorbed by the highly vascularized oromucosal and nasal membranes followed by entering the systemic circulation. Additionally, there is also some gastrointestinal uptake, especially for the oromucosal products (7).

There exists a wide inter-individual and inter-occasion (=intra-individual) variability (IIV and IOV) in the pharmacokinetics of fentanyl (11, 18-22). Several factors may cause variability by influencing absorption, distribution, metabolism, and/or excretion (ADME) (23-25). The intravenous route of administration bypasses the absorption step and is consequently not influenced by factors that affect the absorption rate.

This explains the relatively high IIV and IOV of fentanyl given by the transdermal, oromucosal and nasal route, compared to intravenously administered fentanyl (18, 26). Although fentanyl is mostly dosed by titration nevertheless in specific situations, such as a rotation to fentanyl from another opioid or a change in co-medication, under- or overdosing may occur. Therefore knowledge of factors that influence the variability of fentanyl pharmacokinetics is important. This review aims to study the factors that cause variation in fentanyl pharmacokinetics to increase the understanding of fentanyl pharmacokinetics and its safe use in cancer-related pain. To better understand the mechanism by which the various factors influence pharmacokinetics, we separately describe studies using intravenous fentanyl and studies using other administration routes.

Methods

We performed a review on factors related to pharmacokinetic aspects of immediate release and slow release fentanyl preparations. We searched in PubMed, Cochrane, and Embase (supplement A). The main (Mesh) terms we used were: (fentanyl), (intravenous), (cutaneous), (transdermal), (sublingual), (buccal), (nasal), (transmucosal), (oral), (pharmacokinetics), (biotransformation), (tissue distribution), and (elimination). The search was limited to English or Dutch articles published until July 2014, followed by an update until January 2016.

Additional papers were found by searching the references in selected articles for cross-references. The articles were independently reviewed for eligibility by two authors (E.J.M.K. and M.L.Z.). Studies were included in the analysis if they contained pharmacokinetic parameters (e.g. Area Under the Curve (AUC), Clearance (CL), Time to maximum concentration (Tmax), plasma concentrations), described regularly available fentanyl products and were published in English or Dutch. When the same cohort of patients was described in more than one paper, the paper fitting the inclusion criteria best was chosen. Exclusion criteria were: full text not available, no original research reported, studies in other populations than adults, fentanyl not being the main subject studied, no factors studied in relation to pharmacokinetic parameters, no standard administration route of fentanyl and no pharmacokinetics of fentanyl described. For every publication, we reviewed the type of study, the number of evaluable patients, the administration route, the studied population (e.g. healthy volunteers, cancer patients or peri-operative patients), the number of pharmacokinetic (PK) samples taken per individual (Table 1), blood sample analysis or patch residue analysis, the pharmacokinetic parameters calculated, studied covariate(s) (e.g. age, gender), and the size of the measured effect(s). Drug/molecular target nomenclature is used according to the Concise Guide of Pharmacology (27).

Results

Results of the search

The original search of Pubmed, Embase and Cochrane resulted in 1,543 citations; one extra article was found by checking cross references. Of these 1,544 papers, 31 papers met all the inclusion criteria. The recent update led to one additional paper (13) meeting the strict inclusion criteria, so in total 32 papers were taken into account (**Figure 2**; Prisma figure; **Table 1**). The majority of the studies were performed in healthy volunteers (n=14), about a third in cancer patients (n=11) and the remaining 7 studies in selected other patient populations (patients studied during and after elective surgery, during renal transplantation, patients with rhinitis, and patients with burns). In 13 studies transdermal or intravenous fentanyl was used and in 6 studies oromucosal fentanyl was used.

In total 36 related factors were investigated in these studies. We divided the related factors in four groups: pharmacokinetic drug-drug interactions (**Table 2**), environmental factors (**Table 2**), patient related factors (**Table 3**), and pharmacogenetics (**Table 4**).

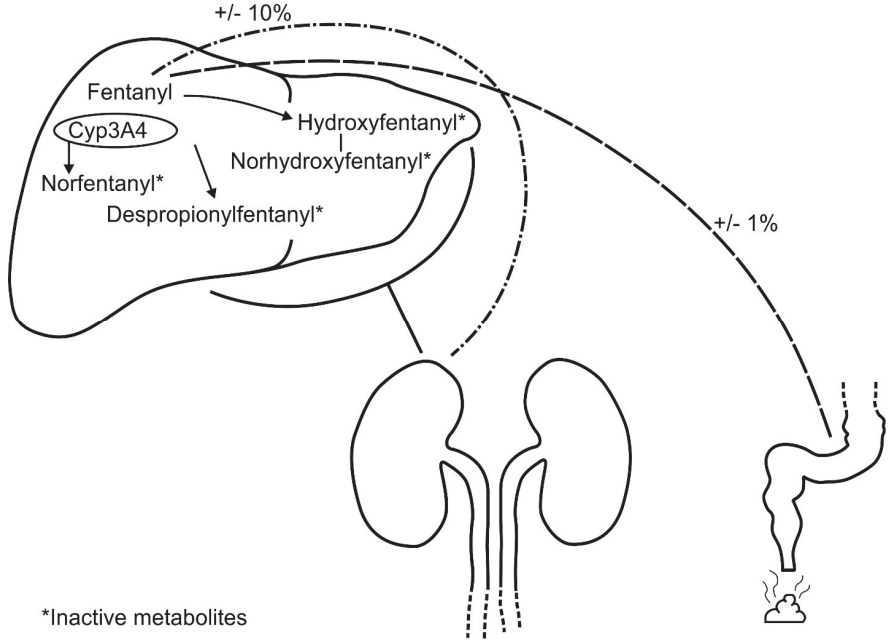

Figure 1 Scheme of metabolism/elimination of fentanyl.

Pharmacokinetic drug-drug interactions

Pharmacokinetic drug-drug interactions are highly relevant in daily clinical practice (28). Especially cancer patients frequently use numerous concomitant drugs during the different phases of their disease. In particular, CYP3A4 inhibitors or inducers may influence fentanyl pharmacokinetics because this iso-enzyme is involved in the conversion of many drugs and also fentanyl is predominantly metabolized by CYP3A4. CYP3A4 inhibitors are divided into strong, moderate and weak inhibitors according their pharmacokinetic effects. By definition, a strong CYP3A4 inhibitor results in a > 5-fold increase in the plasma AUC of a sensitive CYP3A4 substrate and a strong CYP3A4 inducer results in a more than 80% increase in clearance. Moderate inhibitors cause a > 2-fold increase in AUC or a decrease of clearance by 50-80%; and weak inhibitors still cause a clinically relevant > 1.25-fold but < 2-fold increase

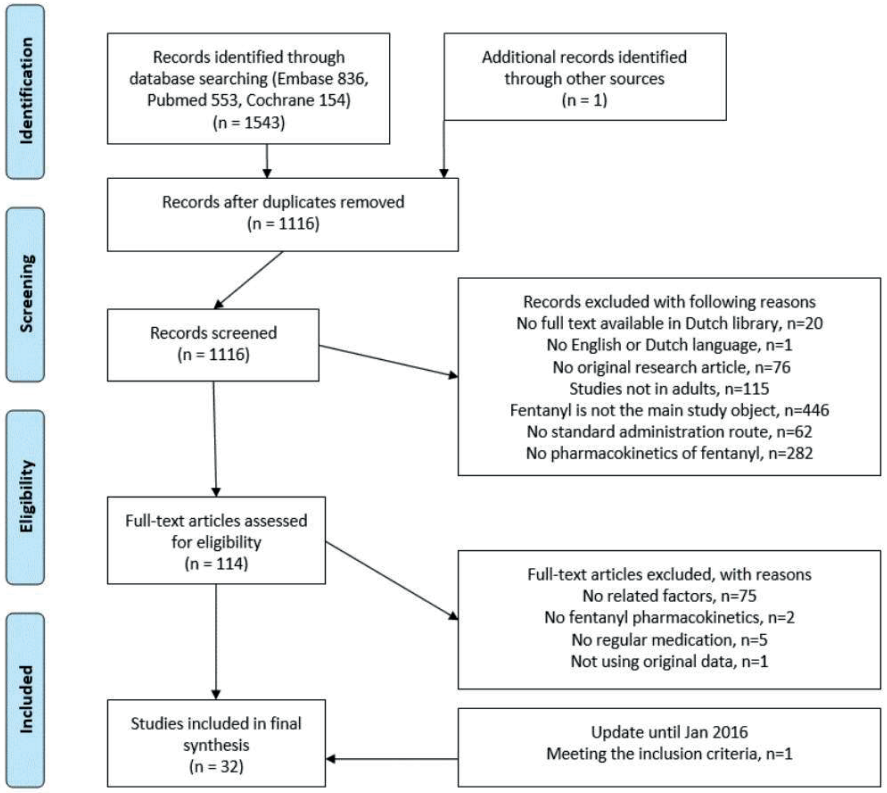

Figure 2 Prisma figure.

in AUC or a decrease of clearance by 20-50% (29). Meanwhile, strong CYP3A4 inducers may lead to a (large) reduction of plasma drug concentrations.

CYP 3A4 inhibitors

The influence of several strong CYP3A4 inhibitors on fentanyl pharmacokinetics has only been studied in healthy volunteers so far. Most studies were performed with intravenous fentanyl and two studies with transmucosal fentanyl. The largest effects were found in volunteers receiving the antiretroviral drug ritonavir and the anti-mycotic compound troleandomycine (12, 30). Concurrent use of ritonavir and (intravenous) fentanyl led to an 2.7-fold increase of the AUC of fentanyl, concurrent use of troleandomycine and (intravenous and transmucosal) fentanyl led to an almost 2-fold increase of the AUC of fentanyl, both drugs compared to fentanyl alone. This effect was similar for the intravenous and transmucosal route of fentanyl, although the standard deviation (sd) of the AUC in the transmucosal group doubled the sd in the group with intravenous fentanyl, reflecting the variable absorption rates for transmucosal fentanyl (12, 30). Use of voriconazole and ketoconazole in combination with fentanyl led to slightly smaller effects on the fentanyl AUC (mean increased AUC of 1.4 times and 1.3 times, respectively) (13, 24). Use of itraconazole showed an non-significant 9% decrease in fentanyl clearance (31). As expected, with the use of moderate CYP3A4 inhibitors, effects were less relevant. Fluconazole in combination with intravenous fentanyl gave a slightly decreased plasma clearance of fentanyl but no significant differences in AUC (24). In another study, grapefruit juice, a strong CYP3A inhibitor, did not lead to changes in pharmacokinetic parameters when combined with transmucosal fentanyl (12).

Interestingly, in all studies, the measured effects on fentanyl pharmacokinetics were relatively small compared to other CYP3A substrates. A pharmacological explanation for some of the small drug – drug effects may be the high extraction ratio of fentanyl. The plasma clearance of fentanyl almost equals hepatic blood flow. For drugs with a high extraction ratio, like fentanyl, a variation of the intrinsic ability to eliminate a drug, results only in marginal change of total clearance. In this case, clearance is mainly affected by liver blood flow (32, 33). However, Ziesenitz et al hypothesized unknown metabolic pathways to explain relatively small effects of ketoconazole on the AUC of fentanyl. They showed a marked inhibition of the formation of norfentanyl with ketoconazole, while the clearance of fentanyl was only modestly decreased. Furthermore, measurements of fentanyl and known metabolites in urine could not retrieve the dose of fentanyl that was administered (13).

Table 1 Overview of the characteristics of included studies

Author	Reference	Year	Country	Study type	N	administration route	Patients	number of PK samples per patient
Ariano	35	2001	Canada	non randomized clinical trial	18	iv	healthy volunteers	14 samples
Ashburn	31	2003	United States	open randomized cross over study	5, 7 and 9	transdermal	healthy volunteers	>36 samples
Baratt	42	2014	Europe (11 countries)cohort	cross sectional study	620	trandermal	cancer patients	1 sample
Bentley	36	1982	United States	non randomized clinical trial	9	iv	post operative patients	19 samples
Darwish	46	2007	United States	non randomized clinical trial	16	buccal	cancer patients	13 samples
Finn	47	2011	United States	non randomized clinical trial	14	buccal	cancer patients	8 samples
Gupta	34	1995	United States	non randomized clinical trial	6, 8 and 11	iv	healthy volunteers	12,27,51 samples
Han	50	2007	Korea	non randomized clinical trial	20	iv	patients with burns	20 samples
Heiskanen	43	2009	Finland	non randomized clinical trial	20	transdermal	cancer patients	5 samples
Holdsworth	37	1994	United States	non randomized clinical trial	16	transdermal	healthy volunteers	47 samples
Ibrahim	21	2003	United States	randomized crossover study	12	iv	healthy volunteers	20 samples per test
Kharasch	38	2004	United States	non randomized clinical trial	24	transmucosal	healthy volunteers	17 samples
Kharasch	22	2004	United States	randomized crossover study	12	transmucosal	healthy volunteers	17 samples
Koehntop	45	1997	United States	non randomized clinical trial	8	iv	patients undergoing renal transplantation	14 samples
Kokubun	24	2012	Japan	cross sectional study	51	transdermal	cancer patients	3 samples
Moore	32	2012	United States	randomized cross over study	20	transdermal	healthy volunteers	18 samples
Nomura	44	2013	Japan	non randomized clinical trial	18	transdermal	cancer patients	8 samples
Olkkola	15	1999	Finland	randomized double blind placebo controlled cross over study	11	iv	healthy volunteers	15 samples
Palkama	23	1998	Finland	randomized double blind cross over study	10	iv	healthy volunteers	14 samples
Parikh	33	2013	United States	randomized crossover study	29	sublingual	healthy volunteers	18 samples
Perelman	48	2013	Canada	randomized crossover study	31	nasal	rhinitis	16 samples
Saari	16	2008	Finland	randomized crossover study	12	iv	healthy volunteers	14 samples
Shomaker	17	2000	United States	non randomized cross over study	6	transdermal	healthy volunteers	25 samples
Singleton	39	1988	United States	non randomized clinical trial	14	iv	post operative patients	19 samples
Solassol	27	2005	France	observational study	108 (507 patches)	transdermal	cancer patients	N.A.
Solassol	40	2005	France	non randomized clinical trial	29	transdermal	cancer patients	2-4 samples
Takashina	51	2012	Japan	non randomized clinical trial	60	transdermal	cancer patients	1 sample
Tanaka	52	2014	Japan	non randomized clinical trial	52	iv	after surgery	1 sample
Thompson	41	1998	United Kingdom	non randomized clinical trial	18	transdermal	post operative patients	18 samples
Van Nimmen	26	2010	Belgium	observational study	68 (498 patches)	transdermal	cancer patients	N.A.
Ziesenitz	25	2013	Germany	prospective randomized cross over study	16	iv	healthy volunteers	20 samples per test
Ziesenitz	18	2015	Germany	randomized crossover study	16	iv	healthy volunteers	20 samples

Table 2 Pharmacokinetic drug interactions & environmental factors

Factor	Administra-tion route	N	Study group	Blood-samples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorp-tion from patch	Other	Significant Effect	Reference
CYP3A4 inhibitors															
troleandomycin	OTFC	12	Healthy volunteers	BS	↑	X	X						Kel, V/F, CL/F	AUC 0-∞ (10.4 +/- 8.9 h*ng/ml) with troleandomycin AUC 0-∞ (5.87 +/- 3.74 h*ng/ml) without troleandomycin	Kharasch (12)
	IV	12	Healthy volunteers	BS	↑	X	X	↓		X	X		Kel ↓	AUC 0-∞ (9.94 +/-3.77 h*ng/ml) with troleandomycin AUC 0-∞ (6.04 +/- 2.19 h*ng/ml) without troleandomycin	Ibrahim (30)
Ritonavir	IV	11	Healthy volunteers	BS	↑			↓		↑	X			AUC 0-∞ (18.1 +/- 6.5 h*ng/ml) with ritonavir AUC 0-∞ (6.6 +/- 3.4 h*ng/ml) without ritonavir CL 5.2 +/- 2.0 ml/min/kg with ritonavir CL 15.6 +/- 8.2 ml/min/kg without ritonavir T1/2: 20.1 +/- 8.4 h with ritonavir T1/2:: 9.4 +/- 4.6 h without ritonavir (P<0.01)	Olkkola (23)
Voriconazole	IV	12	Healthy volunteers	BS	↑			↓		X	X			AUC 0-∞ (8.5 +/- 2.9 h*ng/ml) with voriconazole AUC 0-∞ (6.1 +/- 1.1 h*ng/ml) without voriconazole CL 10.7 +/- 3.0 ml/min/kg with voriconazole CL 14.0 +/- 2.5 ml/min/kg without voriconazole	Saari (24)
Ketoconazole	IV	16	Healthy volunteers	BS	↑	X		↓		X	X			AUC 0-∞ (6.8 +/- 3.4 h*ng/ml) with ketaconazole AUC 0-∞ (5.1 +/-2.5 h*ng/ml) without ketaconazole CL 14.7 +/- 5.6 ml/min/kg with ketaconazole CL 19.0 +/- 6.8 ml/min/kg without ketaconazole	Ziesenitz (13)
Itraconazole	IV	10	Healthy volunteers	BS				X		X	X			No	Palkama (31)
Fluconazole	IV	12	Healthy volunteers	BS	X			↓		X	X			CL 11.6 +/- 3.0 ml/min/kg with fluconazole CL 14.0 +/- 2.5 ml/min/kg without fluconazole	Saari (24)
Grapefruit juice	OTFC	12	Healthy volunteers	BS	X	X	X						Kel, V/F, CL/F	No	Kharasch (12)
CYP3A4 inducers															
Rifampicin	OTFC	12	Healthy volunteers	BS	↓	X	X						Kel ↑, V/F, CL/F ↑	AUC 0- ∞ (2.20 +/- 0.84) with rifampicin AUC 0- ∞ (5.87 +/- 3.74) without rifampicin	Kharasch (12)
Carbamazepine or phenobarbital	Patch	51	Cancer patients	BS	X			↑			X		Ka, tL	Significant influence on CL -> NONMEM analysis CLfenta(L/h)= 3.53x(15-CPS)x (1 +1.38 x *) * CYP3A4 inducers=1, no CYP3A4 inducers=0	Kokubun (34)

Table 2 Continued

Factor	Administra-tion route	N	Study group	Blood-samples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorp-tion from patch	Other	Significant Effect	Reference
Other medication															
Parecoxib	IV	12	Healthy volunteers	BS	X	X	X	X		X	X		Kel	No	Ibrahim (30)
Haloperidol	Patch	68 (498 patches)	Cancer patients	PA								X	Urinary elimination	No	V Nimmen (36)
Morphine	Patch	68 (498 patches)	Cancer patients	PA								X	Urinary elimination	No	V Nimmen (36)
Localization of the patch	Patch	108 (507 patches)	Cancer patients	PA								X		No	Solassol (41)
	Patch	68 (498 patches)	Cancer patients	PA								↑	Urinary elimination	Overall significant influence of site of application (arm, torso, leg) of the patch on transdermal fentanyl delivery (p=0.0011); 7.5% higher delivery efficiency at the arm compared to the leg	V Nimmen (36)
Local heat on patch	Patch	5,7,9	Healthy volunteers	BS	↑	↑	X							AUC 0-4hr 1.22 +/- 0.37 ng*h/ml with heat AUC 0-4hr 0.42 +/- 0.35 ng*h/ml without heat Cmax 0.63 +/- 0.15 ng/ml with heat (after 4 hrs) Cmax 0.24 +/- 0.20 ng ml without heat (after 4 hrs)	Ashburn (45)
	Patch	6	Healthy volunteers	BS	↑	↑	X							AUC 0-4hr 39.1 (9.6-76.8) ng*h/ml with heat AUC 0-4hr 11.3 (0.1-18.0) ng*h/ml without heat Cmax 0.397 (0.14-0.69) ng/ml with heat (after 4 hrs) Cmax 0.126 (0.00-0.18) ng ml without heat	Shomaker(25)
	Patch	20	Healthy volunteers	BS	↑	↑								AUC 0-10hr 1.7 +/- 0.9 ng*h/ml with heat AUC 0-10hr 0.7 +/- 0.5 ng*h/ml without heat Cmax 0.5 +/- 0.3 ng/ml with heat (after 10 hrs) Cmax 0.3 +/- 0.2 ng ml without heat (after 10 hrs)	Moore (46)
Hot/cold beverages	SL	29	Healthy volunteers	BS	X	X	X			X			λz	No	Parikh (47)

Table 2 Continued

Factor	Administra-tion route	N	Study group	Blood-samples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorp-tion from patch	Other	Significant Effect	Reference
Other factors															
Low/high pH beverages	SL	29	Healthy volunteers	BS	X	X	X			X			λz	No	Parikh (47)
Alcohol consumption	Patch (chronic use)	108 (507 patches)	Cancer patients	PA								X		No	Solassol (41)
Smoking	Patch (chronic use)	108 (507 patches)	Cancer patients	PA								X		No	Solassol (41)
Diurnal variation	IV	6, 8, 11	Healthy volunteers	BS	X									No	Gupta (48)

AUC, area under the curve; CL, clearance; CLd, distributional clearance; CL/F, apparent oral clearance; Cmax, maximum concentration; ka, absorption rate constant; IV, intravenous; Kel, terminal elimination rate constant; NONMEM, nonlinear mixed-effect model; OTFC, oral transmucosal fentanyl citrate; SL, sublingual; t_L, lag time; Tmax, time to reach maximum concentration; $T_{½}$, half-life; Vd, volume of distribution; V/F, apparent volume of distribution; X, factor is studied but result not significant; λ_z, terminal disposition rate constant

Table 3 Patient related factors

Factor	Administration route	N	Study group	Bloodsamples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorption from patch	Other	Significant Effect	Reference
Age	IV	18	Healthy volunteers	BS				X	↑		Xa			Higher CLd in elderly (mean 14.59 L/kg/h vs 3.18 L/kg/h)	Ariano (50)
	IV	9	Perioperative patiens	BS				↓		↑	X			Longer t 1/2 in pt >60 yr vs pt <50 yr (945 vs 265 min). Lower CL in pt >60 yr vs pt <50 yr (265 ml/min vs 991 ml/min).	Bentley (26)
	IV	14	Perioperative patients	BS				X		X	↓		Plasma fentanyl concentration ↑	Higher plasma concentration 2 min (mean 7.73 ng/ml vs 4.54 ng/ml) and 4 min (mean 3.26 ng/ml vs 1.78 ng/ml) after infusion in elderly (71-82 yr) vs younger(18-41 yr). Lower VDss in elderly (mean 1.36 l/kg in vs 2.27 l/kg)	Singleton (53)
	OTFC	12	Healthy volunteers	BS	X	X	X						2nd peak Tmax, Kel (1/h), CL/F (l/h), V/F (l)	2nd peak Tmax 1.3 h +/- 0.5 (elderly) vs 1.9+/-0.5 (young)	Kharasch (52)
	Patch	16	Healthy volunteers	BS	↑	X								Mean AUC 0-60hr/PD 2.05ng/ml (67-87 yr) vs 0.88 ng/ml (19-27 yr)	Holdsworth (51)
	Patch (first)	18	Peri/post-operative Patients	BS	X	X	X			X			Half-time ↑	Half-time; in pt 64-82 yr vs pt 25-38 yr (11.1 hrs vs 4.2 hrs)	Thompson (55)
	Patch (chronic use)	108 (507 patches)	Cancer patients	PA								↓		Univariate analysis: Mean fentanyl absorption 55.7% (>75 yr), 63.7% (65-75 yr), 66% (< 65 yr)	Solassol (41)
	Patch	51	Cancer patients	BS	X			X			X		Ka, tL NON-MEM analysis	No	Kokubun (34)
	Patch	68 (498 patches)	Cancer patients	PA								X	Urinary elimination	No	V Nimmen (36)
	Patch	29	Cancer patients	BS/PA				Xb				X	Plasma concentration at steady state	No	Solassol (54)
Gender	Patch	620	Cancer patients	BS									Serum fentanyl ↓and norfentanyl concentrations, MR	Serum fentanyl concentrations ↓ in men, however less than 1% of the variability	Barratt (18)

Table 3 Continued

Factor	Administration route	N	Study group	Bloodsamples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorption from patch	Other	Significant Effect	Reference
	Patch	68 (498 patches)	Cancer patients	PA								X	Urinary elimination	Fentanyl excretion ↑ men / ↓ women (P=0.04)	V Nimmen (36)
	Patch	108 (507 patches)	Cancer patients	PA								X		No	Solassol (41)
Albumin	Patch (first, after IV)	18	Cancer patients	BS									Dose adjusted serum fentanyl concentration ↓	Dose adjusted serum fentanyl concentration (mean) alb < 3.5g/dl alb>3.5 g/dl 15 hr 0.014 0.034 18 hr 0.019 0.028 24 hr 0.018 0.029	Nomura (58)
	Patch	620	Cancer patients	BS									Serum fentanyl and norfen-tanyl concentrations, MR ↓	Inverse association between albumin and serum fentanyl concentrations, explained variability less than 1%. Negative association with MR, explained variability less than 1%.	Barratt (18)
BMI	Patch	20	Cancer patients	BS									Dose adjusted plasma fentanyl concentration ↓	Cachexia (mean BMI 16 kg/m2) vs normal weight (mean BMI 23 kg/m2): At 48 hrs dose adjusted plasma fentanyl concentration 0.014 µg/L vs 0.023 µg/L. At 72 hrs: dose adjusted plasma fentanyl concentration 0.012 µg/L vs 0.024 µg/L.	Heiskanen (59)
	Patch	108 (507 patches)	Cancer patients	PA								X		No	Solassol (41)
	Patch	68 (498 patches)	Cancer patients	PA								X	Urinary elimination	No	V Nimmen (36)
	Patch (first, after IV)	18	Cancer patients	BS									Dose adjusted serum fentanyl concentrations	No	Nomura (58)
	Patch	620	Cancer patients	BS									Serum fentanyl and norfen-tanyl concentrations, MR ↓	Inverse association between BMI and serum fentanyl concentrations, explained variability less than 1%. Negative association with MR, explained variability less than 1%.	Barratt (18)

Table 3 Continued

Factor	Administration route	N	Study group	Bloodsamples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorption from patch	Other	Significant Effect	Reference
	Patch	29	Cancer patients	BS/PA								X	Plasma concentration at steady state	No	Solassol (54)
Renal function															
	IV	8	Patients undergoing renal transplanta-tion	BS				↑			X		T1/2 αβ, Vc, Ke, Kcp, Kpc	BUN > 60mg/dl mean clearance 3.3 ml/kg/min, BUN < 49 mg/dl mean clearance 11.7 ml/kg/min	Koehntop (33)
	Patch	620	Cancer patients	BS									Serum fentanyl and norfen-tanyl concen-trations, MR ↑	↑ MR, explained variability less than 2%.	Barratt (18)
	Patch	68 (498 patches)	Cancer patients	PA								X	urinary elimina-tion	No	V Nimmen (36)
Liver function															
	Patch	51	Cancer patients	BS	↑			↓			X			CPS gr B -> AUC 1.36x larger than CPS gr A CPS gr C -> AUC 3.72x larger than CPS gr A CLfenta(L/h)= 3.53x(15-CPS)x (1 +1.38 x *) * CYP3A4 inducers=1, no CYP3A4 inducers=0	Kokubun (34)
	Patch	68 (498 patches)	Cancer patients	PA								X	urinary elimination ↓	↓ urinary elimination in more severe liver function (p=0.04)	V Nimmen (36)
Mucositis	Buccal	16	Cancer patients	BS	X	X	X							No	Darwish (60)
	Buccal	14	Cancer patients	BS	X	X	X							No	Finn (61)

Table 3 Continued

Factor	Administration route	N	Study group	Bloodsamples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorption from patch	Other	Significant Effect	Reference
Rhinitis +/- treated with oxymethalozine	Intranasal	31	Pts with rhinitis	BS	X	X	X			X				Tmax (min) median (range) Control 17 (10-120) Rhinitis 20 (5-180) Treated (oxymethalozine) 53 (5-180)	Perelman (62)
Hypertrichosis	Patch (chronic use)	507 patches	Cancer patients	PA								X		No	Solassol (41)
Hyperhidrosis	Patch (chronic use)	507 patches	Cancer patients	PA								X		No	Solassol (41)
Burns	IV	20	Pts with burns	BS			↑			X			V	Burned: CL 29.4 mL/min/kg; Unburned 21.0 mL/min/kg	Han (64)

AUC, area under the curve; AUC/PD, AUC/patch duration; BUN, blood urea nitrogen; CL, clearance; Cld, distributional clearance; CL/F, apparent oral clearance; Cmax, maximum concentration; CPS, child pugh score; Half-time, time for plasma concentrations to double after patch application; IV, intravenous; ka, absorption rate constant; Kel, terminal elimination rate constant; Ke, elimination rate constant; Kcp, central to peripheral inter compartmental rate constant; Kpc, peripheral to central inter compartmental rate constant; MR, serum norfentanyl concentration / serum fentanyl concentration; NONMEM, nonlinear mixed-effect model; tL, lag time; Tmax, time to reach maximum concentration; T ½, half-life; Vc, volume of the central compartment; Vd, volume of distribution; V/F, apparent volume of distribution; X, factor is studied but result not significant.

a Vd is specified here as volume of distribution of the peripheral and central compartment

b CL=k0/Css where k0 is the perfusion rate computed from the true amount of fentanyl absorbed over 72hrs and Css is the steady-state fentanyl concentration

Table 4 Pharmacogenetics

Factor	Administration route	N	Study group	Bloodsamples (BS) or residue in patch analysis (PA)	AUC	C_{max}	T_{max}	CL	Cld	T1/2	Vd	Absorption from patch	Other	Significant Effect	Reference
CYP3A5*3	Patch	60	Cancer patients	BS				↓					PCnMAR ↑, MAR, PC,	PCnMAR: *3/*3 group: 2.01 (1.21-2.44);*1/*1 group: 0.82 (0.77-1.25); *1/*3 group: 1.03 (0.80 – 1.74) ng/ml per µg/h/kg (p=0.048 and p=0.021). CL/F: CYP3A5*3/*3 group 28.0 (20.6-55.1); *1/*1 group 67.4 (47.2-78.2); *1/*3 group 58.4 (41.3-75.6) L/h.	Takashina (65)
CYP3A5*3 CYP3A5*1 carriers	IV	52	Postoperative patients	BS									nPC ↑, CLc, CLr, PC	nPC: *3/*3 group: 2.30 (1.09-2.98); *1 group: 1.21 (0.78-1.90) ng/ml per µg/h/kg	Tanaka (66)
ABCB1 C1236T	Patch	60	Cancer patients	BS									MAR, PC, PCnMAR	no	Takashina (65)
SLCO1B1*a1 (genetic wildtype) or *15 (deficient haplotype with altered transport activitiy)	IV	16	Healthy volunteers	BS	X	X		X					AeF, CLren	no	Ziesenitz (35)
CYP3A4*22 en CYP3A5*3	Patch	620	Cancer patients	BS									Serum fentanyl and norfentanyl concentrations, MR	no	Barratt (18)

AUC, area under the curve; AEf, amount of fentanyl excreted in urine within 24 hr after administration; CL, clearance; Cld, distributional clearance; $CL_{ren,}$ renal clearance; CL/F, total clearance of fentanyl; CL_c, creatine clearance (urine); CL_r,renal clearance (urine); MAR, measured absorption rate; MR, serum norfentanyl concentration / serum fentanyl concentration; nPC, plasma concentrations of fentanyl normalized for infusion rate; PC, plasma concentration of fentanyl; PCnMAR, plasma concentration of fentanyl normalized for the measured absorption rate; $T_{½}$, half-life; X, factor is studied but result not significant.

CYP 3A4 inducers

Also the CYP3A4 inducers rifampicin, carbamazepine and phenobarbital were studied for their influence on fentanyl pharmacokinetics (12, 34, 35). None of these studies was performed with intravenously administered fentanyl. Administration of rifampicin to volunteers using transmucosal fentanyl led to a 2.6-fold lower AUC compared to fentanyl alone (12). Carbamazepine and phenobarbital, the used CYP3A4 inducers in a study by Kokubun et al, led to a significantly higher clearance (> 2x as high) of transdermal fentanyl compared to patients not using CYP3A4 (34).

Other

Parecoxib and haloperidol (both CYP3A4 substrates) and morphine (not influenced by CYP3A4) had, as expected, no influence on fentanyl pharmacokinetics (30, 36).

In summary, most strong CYP3A4 inhibitors significantly increased systemic fentanyl exposure while moderate CYP3A4 inhibitors did not. All studied CYP3A4 inducers significantly decreased systemic fentanyl exposure. Although the effects were lower than expected, caution is warranted. Several case reports report interactions between strong CYP3A4 inhibitors and fentanyl with serious outcome like respiratory depression or even death(37-40). In daily clinical practice; use of strong CYP3A4 inhibitors may lead to higher fentanyl plasma levels and probably more (side) effects and use of CYP3A4 inducers may lead to lower fentanyl plasma levels and a risk of inefficient pain relief. Both interactions ask for careful monitoring of patients, especially when the interacting drug is started or stopped during treatment with fentanyl.

Environmental factors

No environmental factors have been studied with intravenous fentanyl.

Localization of the patch

Patients stick fentanyl patches at various sites of the body during chronic treatment. Preferred localizations are the upper arms, thorax, and upper back. These different localizations may potentially influence fentanyl absorption as a result of differences in the thickness of the skin and the subcutaneous fat. Two studies analyzed whether the transdermal absorption from the patch differed between application sites by measuring the fentanyl residue in used patches. No differences in fentanyl absorption were found between patches applied to the arm, shoulder, chest and back (41), while the delivery efficiency of fentanyl was 7.5% lower for patches applied to the leg in comparison to the arm (36).

Local heat or cold

Heat, or raised temperature may increase locally the microcirculation and blood vessel permeability and thereby promote absorption of transdermally delivered drugs. Several case reports have been published about heat induced fentanyl toxicity (e.g sun bathing, warming blanket) in patients using a fentanyl patch (42-44). Three studies investigated the influence of heating the patch on the pharmacokinetics of fentanyl in a controlled setting. Despite some differences in study designs, study outcomes were similar. The AUC and maximum concentration (C_{max}) increased two to four fold when heat (40°C - 44°C) was applied to the patch (25, 45, 46). In another study, the use of hot drinks before taking a dose of fentanyl spray sublingually had no effect on the absorption of fentanyl, neither did the use of cold drinks (47). Other rapid onset fentanyl products like buccal and sublingual tablets have not been studied yet in this setting. Theoretically, local heat may lead to local vasodilatation and probably a higher or faster (peak) concentration of fentanyl.

Other factors

Oromucosally delivered fentanyl is absorbed by transmucosal diffusion. In theory, intake of specific food or drinks before using these fentanyl products may influence fentanyl absorption and, therefore, pharmacokinetics. Experimental pre-treatment with **high pH beverages** (solution of sodium bicarbonate in water) **or low pH beverages** (e.g. Coca-Cola or Sprite) has been studied but did not influence absorption of fentanyl from a sublingual spray (47).The influence of both **alcohol and smoking** on the pharmacokinetics of fentanyl was studied in one study analyzing fentanyl residue in patches (41). No significant influence of both factors on fentanyl absorption rate was found. Finally, the effect of **diurnal variation** on fentanyl pharmacokinetics was studies during 3 consecutive days; no influence on its pharmacokinetics was found. Whether there is no effect at all of chrono-pharmacology on fentanyl pharmacokinetics, needs to be shown in future studies (48).

In summary, only local heat applied to transdermal fentanyl patches significantly increased the AUC of fentanyl. In daily clinical practice patients using fentanyl patches should be aware of the risk of a fentanyl intoxication in situations like fever, sun bathing, using a warming blanket and doing heavy exercise (42, 44, 49). All the other environmental factors studied did not significantly influence fentanyl pharmacokinetics.

Patient related factors

Various patients characteristics may potentially influence the pharmacokinetic IIV of fentanyl. All studied characteristics are summarized below, however not all characteristics are studied as extensively as others.

Age

Ageing is a multifactorial continuous process which may result in changed body composition, impaired renal function, decreased muscle tissue and comorbidity leading to changes in all different phases of pharmacokinetics. Age was studied widely as a potential factor influencing fentanyl pharmacokinetics (26, 34, 36, 41, 50-55). The intravenous (26, 50, 53), transdermal (34, 36, 41, 51, 54, 55) and transmucosal (52) administration routes were studied. Even though all these studies investigated the relation between fentanyl pharmacokinetics and age, the methods used and pharmacokinetic parameters calculated were quite miscellaneous, making it difficult to draw solid conclusions.

Overall, 5 studies were explicitly designed to investigate pharmacokinetic differences between elderly and younger patients (26, 51-53, 55). Two of these studies used intravenous fentanyl, both performed in a perioperative setting (26, 53). In one of the perioperative studies, clearance was found to be significantly lower in older patients (26). Similar conflicting results were found in the three studies using other administration routes: in one of the two studies using transdermal fentanyl a higher AUC was found for elderly patients (51), the study on transmucosal fentanyl found no effect of age (52).

Although lower fentanyl clearance in elderly patients may be explained by lower plasma albumin, decreased hepatic blood flow or decreased renal function, the exact reason remains unclear in the studies on intravenously administered fentanyl (56). Especially confounding factors during surgery as the use of anesthetics and effects on (portal) blood flow during abdominal surgery may contribute to results in the perioperative studies (26, 53, 55).

In other studies age was one of several factors studied (in multivariate analyses) (34, 36, 41, 54). In all studies transdermal fentanyl was used. These studies did not show significant effects of age on various pharmacokinetic parameters, except for the study of Solassol (41). A small difference of 10% in mean fentanyl absorption (residue in patch) was found between patients $>$ 75 years compared to patients $<$ 65 years, with lower absorption rates for elderly patients. This variation is most likely too small to cause different clinical effects between elderly and younger patients in daily clinical practice.

In summary, although ageing may influence fentanyl pharmacokinetics, it is difficult to draw solid conclusions. There is at least a risk on lower clearance and thus higher AUC's in elderly. Therefore, our advice is to titrate fentanyl cautiously in elderly patients.

Gender

The effect of gender on fentanyl PK is only studied in patients using transdermal patches. Gender may influence fentanyl pharmacokinetics by a higher CYP3A4 activity in women compared to men and by differences in body composition between men and women (57). Three studies investigated whether gender influences the pharmacokinetics of fentanyl patches; two analyzed residue in patches while one studied pharmacokinetics by serum samples (18, 36, 41). In these studies gender did not influence absorption from the patch (36, 41). Men had lower serum fentanyl concentrations than women but the influence of gender was quite small as less than 1% of the inter-individual variations was explained by gender (18). Gender may influence elimination, suggested by a higher average urinary fentanyl excretion for men than for women (36). It is questionable however, how important this is because fentanyl is mostly metabolized in the liver, and only 10% is excreted unchanged in the urine. So, based on these studies there is no reason for gender specific dose modifications of fentanyl.

Albumin

Fentanyl is highly lipophilic and binds to plasma proteins (e.g albumin, alpha-1-acid glycoprotein). The effect of hypoalbuminemia was studied in two studies with a different design, both in patients using a fentanyl patch (18, 58).

In the study by Nomura et al, serum fentanyl concentrations were measured every 3 hours during conversion from intravenous fentanyl to transdermal patch(es). After 6 hours the intravenous fentanyl was stopped. The patients with an albumin $<$ 3.5 g/dL had significantly reduced dose-adjusted serum fentanyl concentrations at 9-24 hours after application of the patch compared to patients with an albumin $>$ 3.5 g/dL. The authors suggest lower absorption from the patch in patients with low plasma albumin concentrations (58). Barrett et all showed no clinically relevant influence of albumin on fentanyl plasma concentrations in a large cross-sectional study in patients using transdermal fentanyl (18).

Neither study measured the free unbound fraction of fentanyl to better clarify the influence of plasma proteins on fentanyl pharmacokinetics. Also the cause of hypo-albuminemia (cachexia, liver failure) may influence fentanyl pharmacokinetics. The cause of the hypoalbuminemia may predict also if fentanyl pharmacokinetics are influenced in other ways than just absorption. A new study with intravenous fentanyl would be helpful to sort this out. In daily practice, it is important to realize that higher doses of transdermal fentanyl may be needed to reach adequate pain relief when albumin levels decrease during phases of disease.

Body Mass Index (BMI)

The Body Mass Index (BMI) or quetelet-index (QI), is the most widely used index to indicate under- or overweight of people. It is calculated by weight (in kilogram) divided by length in meter squared. As BMI is related to body composition and the thickness of subcutaneous fat, transdermal fentanyl absorption and tissue distribution is expected to be larger in patients with high vs low BMI. Several studies investigated

the effect of BMI on fentanyl pharmacokinetics. Intravenous fentanyl was not studied, all studies included cancer patients using patches (18, 36, 41, 54, 58, 59). Only Heiskanen et al found a statistically significant difference in plasma fentanyl concentration between cachectic patients and normal weight patients (59). At 48 and 72 hours after applying the patch, plasma fentanyl concentrations were almost twice as low in cachectic patients compared to normal weight patients . A limiting factor of this study was that serum albumin concentrations were not measured in this study. (59). Nomura et al. found no differences in absorption between low weight and normal weight patients during the first 24 hours after conversion from intravenous to transdermally delivered fentanyl (58). In that study median serum albumin concentrations were similar in the low weight and normal weight patient groups. Thus, the lower plasma fentanyl concentrations found by Heiskanen et al in the cachectic patients might be explained by hypoalbuminemia (59).

Three studies analyzed the fentanyl residue in patches. Neither of these studies showed significant differences between different BMI groups (36, 41, 54).

BMI is only studied in patch studies and not in intravenous and/or oromucosal administration routes. So it is unclear of besides possible influence on absorption it will also influence other parts of fentanyl metabolism.

So, one study found a significant lower fentanyl concentrations in extremely cachectic patients using fentanyl patches. Other studies did not find differences between low weight and normal weight patients. In daily practice physicians should be aware that fentanyl patches may be less effective in cachectic patients.

Renal function

Although fentanyl is mainly metabolized in the liver into the inactive metabolite norfentanyl, about 10% of both compounds are excreted by the kidneys. Three studies investigated whether kidney function influences fentanyl pharmacokinetics (18, 33, 36). Koehntop et al studied intravenous fentanyl clearance in a specific setting, 8 patients undergoing renal transplantation for terminal kidney dysfunction. They found that a blood urea nitrogen (BUN) > 60mg/dL was associated with a lower clearance compared to a BUN < 49mg/dL. This effect probably reflected the heterogeneity in dialysis status, renal failure induced abnormalities and probably most important dynamic changes during surgery in this small study group (33). In a study in which the excretion of fentanyl by the urine was measured in patients using fentanyl patches, elimination of fentanyl was not influenced by moderate to severe renal impairment (36). In this study, 20% of the patients had moderate or severe renal impairment defined as glomerular filtration rate (GFR) between 15 and 59 mL/min/1.73m^2. GFR and kidney disease were also part of the multivariate analysis of Barrett et al (18). GFR was not associated with serum fentanyl concentrations. In daily practice there is no reason to adjust fentanyl dose depending on renal status.

Liver function

Fentanyl is mainly metabolized in the liver by CYP3A4 into inactive metabolites and therefore, it is expected that liver disease will impair fentanyl clearance. Intravenous fentanyl is not studied; all studied report on transdermal fentanyl. In a study including patients with various degrees of liver failure, a clinically significant effect on pharmacokinetics was found. In this study of Kokubun et al, Child Pugh Score was used to describe the severity of liver disease. Child Pugh score A is defined as mild (5-6 points), B as moderate (7-9 points) and C as severe (10-15 points) liver disease. The study showed that in severe liver failure, the AUC of fentanyl was increased; a higher Child Pugh Score was related to a lower clearance of fentanyl. Severe liver failure led to a 7 times lower clearance of fentanyl compared to mild liver failure(34). The finding of clearly decreased urinary elimination of fentanyl in liver failure in a study measuring fentanyl excretion in urine was in line with the Kokubun study (36). Both studies showed that an impaired liver function influences fentanyl pharmacokinetics importantly. Therefore, fentanyl doses have to be adjusted when patients develop liver failure.

Mucositis

Mucositis is a painful inflammation and ulceration of the mucosa of the gastrointestinal tract. It may develop everywhere along this tract. Oral mucositis refers to mucositis of the mouth and occurs often in cancer patients during treatment with chemotherapy or radiation. Nowadays several rapid-onset products of fentanyl are available (7). The sublingual and buccal rapid-onset fentanyl products are absorbed by the oral mucosa followed by systemic delivery. Changes in the mucosal integrity due to mucositis have been hypothesized to influence the absorption of fentanyl. Two studies have been performed comparing the pharmacokinetics of bucally delivered fentanyl products in cancer patients with and without mucositis (60, 61). Both studies only included patients with a clinical grade 1 mucositis. Grade 1 mucositis consists of erythema, painless ulcers or mild soreness (CTCAE criteria). In neither study a statistically significant difference in the pharmacokinetic parameters C_{max}, T_{max} and AUC was found between the patients with and without mucositis, although one of the studies showed a trend towards a higher AUC (median 2.05 ng/h/ml vs 1.55 ng/h/ml) in patients with mucositis (60). Whether transmucosally fentanyl products can be safely and effectively used in regular doses in patients with more severe mucositis needs to be studied in adequately powered studies including patients with more severe and, in that case, painful mucositis.

Rhinitis

Rhinitis is defined as irritation and inflammation of mucosa inside the nose caused by allergens, viruses, bacteria or irritants. In one study the effect of rhinitis and treatment

with oxymetazoline was studied. In patients prone to develop allergic rhinitis pharmacokinetics of intranasal fentanyl were measured in periods with or without rhinitis and with or without treatment with oxymetazoline. Rhinitis per se did not influence fentanyl pharmacokinetics, but the use of oxymetazoline reduced the C_{max} with almost 50%, most likely caused by vasoconstriction by oxymetazoline. Patients using intranasal fentanyl should not use concomitant local vasoconstrictives because of dramatically reduced concentrations of fentanyl probably leading to insufficient pain relief (62).

Hypertrichosis and hyperhidrosis

Hypertrichosis is an abnormal hair growth over the body and hyperhidrosis is a disorder marked by excessive sweating. Both factors are studied in a patch study analyzing the residue in patches. Both factors could potentially influence local adherence of the patch on the skin. However, neither factor influenced absorption in univariate analyses. Unfortunately, the severity of the hypertrichosis and hyperhidrosis --especially at the location of the fentanyl patch-- were not described in the publication, making it difficult to interpret these findings (41).

Burns

Patients with severe burns start in a hypodynamic state immediately after the accident and end up in a hypermetabolic state represented by an increased cardiac output and reduced systemic vascular resistance (63). Due to these hemodynamic changes, pharmacokinetic characteristics may be influenced. One study investigated the influence of severe burns on the pharmacokinetics of intravenous fentanyl in patients scheduled for burn-related surgery in their hyperdynamic phase. Patients with major burns (mean 49% +/-3% burn of body surface area) were compared to demographically matched controls. In the patients with burns, clearance of fentanyl was about 44% higher than in the control group. This higher clearance may be caused by increased cardiac output and the resultant increased hepatic blood flow (64). Although severe burns have significant impact on fentanyl clearance this is not a common scenario for most cancer patients using fentanyl patches.

Pharmacogenetics

The effects of genetic variation on fentanyl pharmacokinetics are studied in only a few trials so far (18, 35, 65, 66). Two studies used intravenous fentanyl (35, 66), the other studies used transdermal fentanyl. As mentioned earlier, fentanyl is thought to be mainly metabolized by CYP3A4. Of note, CYP3A5 contributes to CYP3A-dependent drug clearance and in such a way may lead to changes in fentanyl pharmacokinetics as well (67).

Patients with the *CYP3A5*3* gene single nucleotide polymorphism (SNP) had about a 2-fold higher fentanyl plasma concentration normalized by measured

absorption rate than patients with the wildtype (**1*1*) gene polymorphism and the patients with the heterozygous (**1*3*) gene polymorphism (65, 66). The total clearance of fentanyl is also 30-50% lower for the **3*3* group compared to the other two groups (65). Though Barrett et al. found no influence of *CYP 3A5*3* or the recently discovered *CYP3A4*22* SNP on serum fentanyl concentrations(18). This discrepancy may (partly) be caused by the timing of taking blood samples. In the study by Barrett et al. one random sample was taken during fentanyl use, while the samples in the two other studies for all patients were exactly timed (65, 66). Next to enzymes, also the efflux drug transporter ABCB1 (P-glycoprotein) was studied. This protein is responsible for the transport of fentanyl through the blood brain barrier (a.o.) (68). SLCO1B1 is another protein responsible for transport (35). Variations in genes coding for these proteins may therefore influence fentanyl pharmacokinetics. However both the ABCB1 1236 polymorphisms and SLCO1B1* and *15 polymorphisms were not found to influence fentanyl pharmacokinetics (35, 65). So, CYP3A5 polymorphisms may influence, the fentanyl PK. However, more research is needed before implementing genotyping in clinical practice.

Discussion and future perspectives

In this review an overview of currently studied factors in relation to the pharmacokinetics of fentanyl is provided. Awareness of these different factors that influence fentanyl pharmacokinetics is important to prevent over and underdosing of fentanyl, leading to intoxication or insufficient pain relief. This is especially relevant in case of opioid rotation. The most pronounced effects on fentanyl PK can be expected when given in combination with strong CYP3A inhibitors, or inducers or in case of impaired liver function. In these cases, patients should be monitored closely especially with changes in the prescription of (combinations of) strong CYP3A4 inhibitors and inducers, or in case of deteriorating liver function.

Another important factor leading to clinically relevant increases in fentanyl exposure, is the adding of local heat to a fentanyl patch. This directly promotes the absorption of fentanyl and should therefore be avoided. (12, 13, 23-25, 30, 31, 34, 35).

Conflicting results were reported for the factors age, BMI and gender. This is particularly due to the enormous heterogeneity of the included populations (healthy volunteers, (peri-) operative patients and cancer patients), the methods used in these studies with only a minority of the studies performed with intravenous fentanyl and the studied pharmacokinetic outcome parameters. Many studies did not report clinically important pharmacokinetic parameters like AUC, T_{max} and $T_{1/2}$. Furthermore, power analyses for prespecified pharmacokinetic endpoints were not described in the majority of studies. The studies to investigate the effect of BMI and gender were

done in patients/volunteers using patches and in this way absorption may be the major cause of interpatient variability. A factor influencing clearance is in this case less easy to detect.

In general, the prevalence of cancer is especially high in elderly people. More knowledge on the effect of age in relation to fentanyl PK would therefore be helpful in adequate dosing of fentanyl. Other common patient variables like smoking habits and use of alcohol are also sparsely studied. These factors are prone to change during different phases of disease. Possibly, these factors explain partly the wide variety in fentanyl PK. Fentanyl is widely used for cancer-related pain, as many patients and health care professionals prefer a patch for drug delivery for reasons of convenience. The patch is especially appropriate for specific patient populations like patients with swallowing disorders, bowel obstruction and patients at the end of life. However, typical problems in these specific patient populations are cachexia and dehydration. The influence of these factors on fentanyl uptake and clearance is still largely unclear. Cancer patients usually use several drugs for other diseases or intercurrent problems (e.g. infectious problems), but also medication to treat side effect of cancer therapies. Most of these drugs are hardly studied for their effects on fentanyl clearance. Therefore, it is unclear whether commonly used co-medication like clarithromycin, verapamil, or aprepitant (particularly used by cancer patients to treat chemotherapy induced nausea and vomiting) influence fentanyl pharmacokinetics. Also (strong) CYP3A4 inducers are sparsely studied, e.g for phenytoin or St. John's wort the influence is currently unclear, although these effects are probably clinically relevant.

Of note, most published studies were performed in patients using a fentanyl patch or intravenous fentanyl. Nowadays, several other fentanyl products are available; the Rapid Onset Opioids (ROOs), for the treatment of breakthrough pain. Only 6 studies in this review studied one of the ROO's (12, 47, 52, 60-62) and just two studies were performed in cancer patients (60, 61). Although the metabolism of fentanyl is the same for all fentanyl products, transmucosal absorption may be influenced by local factors like mucositis or dry mouth. The studies in this review that investigated mucositis included only patients with a low grade mucositis and no influence on fentanyl pharmacokinetics was found (60, 61). Unfortunately, patients with painful (higher grade) mucositis were not included. For this reason, at the Erasmus MC Cancer Institute we are currently performing a pharmacokinetic study with sublingual fentanyl in patients with at least grade 2 mucositis caused by radiotherapy in combination with cisplatin or cetuximab in head and neck cancer patients (www.trialregister.nl; study number NTR4995).

Furthermore, we found no studies on the effect of xerostomia on the absorption of fentanyl using ROOs for sublingual and buccal use. Although patients are advised rinsing their mouth with water before taking the drugs, evidence on a protective effect in dry mouth is not available in literature. Since dry mouth is a common side effect , e.g. in patients using opioids and patients formerly treated for head and neck cancer, studies on the pharmacokinetics and clinical effects of ROOs in these patient groups are awaited.

A few trials included in this study showed that a part of the variation in fentanyl concentration can be explained by the CYP3A5*3 SNP. However, not all studies showed the same effect and the effects of the investigated polymorphisms in relation to fentanyl pharmacokinetics were small and do not support routine genotyping in clinical practice.

An important limitation of this study is that we only investigated pharmacokinetic variability of fentanyl. The investigated covariates were not correlated to pharmacodynamic effects in terms of side effects and pain relief. We assumed that changes of more than 25-30% lead to clinical relevant effects. However, a clear relation between fentanyl pharmacokinetics and the incidence and severity of fentanyl induced side effects has not yet been demonstrated.

We have chosen to describe the main characteristics of the included studies without using a specific tool to assess the quality of the selected studies (**table 1**). In this review, we aimed to describe as many as possible potential factors influencing fentanyl pharmacokinetics independently of the kind of study or outcome. This could be a limitation of our search, but provides the most broad overview currently possible in the field of fentanyl pharmacokinetics.

In summary, in this review we found several factors influencing fentanyl pharmacokinetics, but we still cannot completely explain the wide intra- and inter-patient variability. (11, 18-21, 69). During the next years, we hope and expect that new data will become available to further unravel the complex pharmacokinetics of fentanyl in both cancer and non-cancer related pain. In our view, prospective research on fentanyl pharmacokinetics should be more focused on cancer patients using various fentanyl products, during several phases of the disease trajectory (curable and non-curable disease) and on the relation between pharmacokinetics and clinical effects, both pain relief and side effects.

Appendix

Supplement A

Embase

(Fentanyl:de,ab,ti OR Phentan*:ab,ti OR Sublimaze:ab,ti OR Fentora:ab,ti OR 'R 4263':ab,ti OR R4263:ab,ti OR Duragesic:ab,ti OR Durogesic:ab,ti) AND (Pharmacokinetic*:de,ab,ti OR kinetic*:de,ab,ti OR ((absor*:de,ab,ti OR (biological NEXT/1 transport*):de,ab,ti OR (tissue NEXT/1 distribut*):de,ab,ti OR biotransform*:de,ab,ti OR elimin*:ab,ti OR toxic*:de,ab,ti) AND (dosage*:de,ab,ti OR dosis:de,ab,ti OR dosing:de,ab,ti OR doses:de,ab,ti OR dose:de,ab,ti OR metabol*:de,ab,ti))) AND (transderm*:de,ab,ti OR (trans NEXT/1 derm*):de,ab,ti OR oral:de,ab,ti OR buccal:de,ab,ti OR subling*:de,ab,ti OR (sub NEXT/1 ling*):de,ab,ti OR nasal:de,ab,ti OR subcutan*:de,ab,ti OR intraven*:de,ab,ti OR (trans NEXT/1 muc*):de,ab,ti OR (sub NEXT/1 cutan*):de,ab,ti OR (intra NEXT/1 ven*):de,ab,ti OR transmuc*:de,ab,ti OR (method* NEAR/3 administ*):de,ab,ti OR 'drug administration route'/exp) NOT ((animals)/lim NOT (humans)/lim) AND ((English)/lim OR (71)/(71)lim) NOT ((child)/lim NOT (adult)/lim) NOT ((editorial)/lim OR (letter)/lim OR (review)/lim OR (conference abstract)/lim OR (conference paper)/lim OR (conference review)/lim)

PubMed

(Fentan*(tw) OR Phentan*(tiab) OR Sublimaze(tiab) OR Fentora(tiab) OR R-4263(tiab) OR R4263(tiab) OR Duragesic(tiab) OR Durogesic(tiab))
AND
(Pharmacokinetic*(tw) OR kinetic*(tw) OR
((absor*(tw) OR biological transport*(tiab) OR tissue distribut*(tw) OR biotransform*(tw) OR elimin*(tiab) OR toxic*(tw)) AND (dosage*(tiab) OR dosis(tiab) OR dosing(tiab) OR doses(tiab) OR dose(tiab) OR metabol*(tiab))))
AND
(transderm*(tw) OR trans derm*(tw) OR oral(tw) OR buccal(tw) OR subling*(tw) OR sub ling*(tw) OR nasal(tw) OR subcutan*(tw) OR sub cutan*(tw) OR intraven*(tw) OR intra ven*(tw) OR transmuc*(tw) OR trans muc*(tw) OR methods of administ*(tw) OR administration method*(tw) OR Drug Administration Routes(mesh))
NOT (animals(mesh) NOT humans(mesh))
NOT (children(mesh) NOT adults(mesh))
AND (english(lang) OR Dutch(lang))
NOT (letter(pt) OR review(pt) OR editorial(pt))

Cochrane

(Fentanyl:ab,ti OR Phentan*:ab,ti OR Sublimaze:ab,ti OR Fentora:ab,ti OR 'R 4263':ab,ti OR R4263:ab,ti OR Duragesic:ab,ti OR Durogesic:ab,ti) AND (Pharmacokinetic*:ab,ti OR kinetic*:ab,ti OR ((absor*:ab,ti OR (biological NEXT/1 transport*):ab,ti OR (tissue NEXT/1 distribut*):ab,ti OR biotransform*:ab,ti OR elimin*:ab,ti OR toxic*:ab,ti) AND (dosage*:ab,ti OR dosis:ab,ti OR dosing:ab,ti OR doses:ab,ti OR dose:ab,ti OR metabol*:ab,ti))) AND (transderm*:ab,ti OR (trans NEXT/1 derm*):ab,ti OR oral:ab,ti OR buccal:ab,ti OR subling*:ab,ti OR (sub NEXT/1 ling*):ab,ti OR nasal:ab,ti OR subcutan*:ab,ti OR intraven*:ab,ti OR (trans NEXT/1 muc*):ab,ti OR (sub NEXT/1 cutan*):ab,ti OR (intra NEXT/1 ven*):ab,ti OR transmuc*:ab,ti OR (method* NEAR/3 administ*):ab,ti)

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3

Bioanalytical methods for the quantification of hydromorphone, fentanyl, norfentanyl, morphine, morphine-3ß-glucuronide and morphine-6ß-glucuronide in human plasma

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Abstract

The aim of this study was to develop an assay for the quantification of hydromorphone, morphine, fentanyl and the metabolites norfentanyl, morphine-3ß-glucuronide and morphine-6ß-glucuronide in human plasma to support pharmacokinetic studies investigating the large interpatient variability in response to opioid treatment.

For the quantitation of hydromorphone, morphine, fentanyl and its metabolite norfentanyl aliquots of 200 µL human potassium EDTA plasma were deproteinized with deuterated internal standards in a mixture of acetonitrile and acetone, followed by a liquid-liquid extraction with 4% ammonium hydroxide and ethyl acetate. Morphine-3ß-glucuronide and morphine-6ß-glucuronide were extracted by a solid phase extraction using 10 mM ammonium carbonate pH 8.8 and a deuterated internal standards solution. Morphine, hydromorphone, fentanyl and norfentanyl were separated on an Aquity UPLC® BEH C18 column 1.7 µm, 100 mm x 2.1 mm at 50°C. Separation, was achieved on a gradient of methanol with an overall run time of 6 minutes. The compounds were quantified by triple-quadrupole mass spectrometry in the positive ion electrospray ionization mode. Morphine-3ß-glucuronide and morphine-6ß-glucuronide were separated on a VisionHT C18-P; 3µm 2.1x50 mm, column at 40°C on a gradient of acetonitrile, with an overall run time of 10 minutes. Both methods were precise and accurate, with within-run and between-run precisions within acceptable limits and accuracy ranging from 84.0 to 105.5%. The methods were successfully applied to support clinical pharmacological studies in patients treated with opioids for the treatment of moderate to severe cancer-related pain.

Introduction

Many patients with cancer at all stages of disease suffer from mild to severe pain requiring treatment with opioids (1). Unfortunately, the response to opioid treatment is highly variable among patients. Some patients suffer from severe side effects and other patients have inadequate pain relief despite increasing opioid doses (2,3).

An important factor responsible for the variation in response might be the (largely unexplained) variability in pharmacokinetics (4,5). To reduce the incidence of severe opioid related side effects and inadequate pain relief, factors explaining pharmacokinetic variability need further investigation. This requires a validated method to measure opioids in plasma. In this study, the opioids morphine, fentanyl and hydromorphone are investigated.

Morphine undergoes extensive glucuronidation to the two main morphine metabolites, morphine-3ß-glucuronide (M3G) and morphine-6ß-glucuronide (M6G) (6,7). M3G is thought to be inactive, since administration of M3G to healthy volunteers did not result in any clinical effect (8). M6G however, is able to contribute to the analgesic effects. This is especially relevant in patients with renal impairment which may result in M6G accumulation (9,10).

Fentanyl is a synthetic opioid approximately 75 - 100 times more potent than morphine and is mainly metabolized to the inactive metabolite norfentanyl (11). Advantages that promote its use are the various patient friendly administration routes, the relatively low incidence of gastrointestinal related side effects and its recommended use in patients with renal impairment (12).

Last, but not least, hydromorphone is a derivate of morphine and approximately 5 times more potent compared to morphine (13). If inadequate pain relief is reached with the more common opioids fentanyl, morphine and oxycodone, hydromorphone can be considered.

Especially opioid rotations are moments at risk for severe opioid toxicity or inadequate pain relief (14). The opioid dose conversions schemes give some direction for dose adjustments but the optimal time interval between stopping the prior opioid and starting a new opioid is unknown and needs further pharmacokinetic evaluation.

Several multi-analyte assays for the simultaneous detection of opioids and metabolites in human plasma or serum are described (15, 16). A disadvantage of these methods is the use of laborious and time-consuming solid phase extraction. The method described by Eckart et al. includes several opiods and metabolites, but the detection limit for fentanyl and norfentanyl was validated at a concentration of 1 ng/mL which is not sensitive enough for use in pharmacokinetic studies, especially when fentanyl is administered sublingually or change in opioid regimen with subcutaneous and transdermal fentanyl in cancer patients (17). Musshoff et al. described also a method for the detection of several opioids by using a solid-phase

extraction, but they use a large sample size of 1-mL and also the detection limit for fentanyl and norfentanyl is 1 ng/mL.

The aim of this study was to develop sensitive bioanalytical assays to measure hydromorphone, fentanyl, norfentanyl, morphine, morphine-3ß-glucoronide and morphine-6ß-glucuronide in human plasma.

1 Experimental

1.1 Chemicals

Hydromorphone, fentanyl, norfentanyl oxalate, morphine, morphine-3ß-glucuronide, morphine-6ß-glucuronide and the deuterated internal standards hydromorphone-d6, fentanyl-d5, norfentanyl-d5 oxalate, morphine-d6, morphine-3ß-glucuronide-d3 and morphine-6ß-glucuronide-d3, were obtained from Cerilliant (Round Rock, TX, USA). Acetonitrile, methanol, water and ethyl acetate were purchased from Biosolve BV (Valkenswaard, The Netherlands). Dimethylsulphoxide and ammonium formate were from Sigma-Aldrich (Zwijndrecht, The Netherlands). Formic acid and ammonium hydroxide were obtained from J.T. Baker (Deventer, The Netherlands) and 2-propanol, acetone and ammonium carbonate from Merck (Darmstadt, Germany). Blank potassium EDTA plasma was purchased from Biological Specialty Corporation (Colmar, PA, USA). All chemicals were of analytical grade or higher.

1.2 Preparation of stock solutions, calibration standards and quality control samples

Hydromorphone, morphine, fentanyl and norfentanyl oxalate stock solutions were provided as ready to use solutions with a concentration of 1 mg/mL free base in methanol. Morphine-6ß-glucuronide was provided as ready to use solution with a concentration of 0.1 mg/mL free base in methanol/water (1:1, v/v) and morphine-3ß-glucuronide was provided as ready to use solution with a concentration of 1 mg/mL free base in methanol / 0.05% NaOH (w/v). The deuterated internal standard stock solutions were provided as ready to use solutions with a concentration of 0.1 mg/mL for hydromorphone-d6, fentanyl-d5, norfentanyl-d5 oxalate in methanol, while morphine-d6 was provided as ready to use solution with a concentration of 1 mg/mL free base in methanol. Morphine-3ß-glucuronide-d6 and morphine-6ß-glucuronide-d6 were provided as ready to use solutions with a concentration of 0.1 mg/mL in methanol / 0.05% NaOH (w/v) and methanol / water (1:1, v/v) respectively. All stock solutions were stored at T<-70°C. Internal standard stock solutions of morphine-d6, hydromorphone-d6, fentanyl-d5 and norfentanyl-d5 oxalate were diluted in acetonitrile, resulting in an internal standard solution containing 125 ng/mL morphine-d6, 12.5 ng/mL hydromorphone-d6, 0.5 ng/mL fentanyl-d5 and 10 ng/mL norfentanyl-d5 oxalate respectively and stored at T<8°C for a maximum of 3 months. Internal standard stock solutions of morphine-3ß-glucuronide-d3 and morphine-6ß-glucuronide-d3 were prepared by dilution of stock solutions in acetonitrile/water (1:1, v/v) resulting in an internal standard solution containing 100 ng/mL and stored at T<8°C for a maximum of 3 months.

Calibration standards were prepared freshly on the day of analysis, in duplicate, by addition of 10-µL aliquots of appropriate dilutions of hydromorphone, morphine, fentanyl and norfentanyl stock solution in acetonitrile/DMSO (1:1, v/v) to 190-µL aliquots of human potassium EDTA plasma at the following concentrations: 1.00, 2.50, 5.00, 10.0, 25.0, 50.0, 90.0, and 100 ng/mL as free base for hydromorphone and morphine, 0.200, 0.500, 1.00, 2.00, 5.00, 10.0, 18.0 and 20.0 ng/mL as free base for norfentanyl and 0.100, 0.250, 0.500, 1.00, 2.50, 5.00, 9.00 and 10.0 ng/mL as free base for fentanyl. Calibration standards for morphine-3ß-glucuronide and morphine-6ß-glucuronide were prepared freshly on the day of analysis, in duplicate, by addition of 10 µL aliquots of appropriate dilutions of stock solution in acetonitrile/DMSO (1:1, v/v) to 190-µL aliquots of human potassium EDTA plasma at the following concentrations: 10.0, 25.0, 50.0, 100, 250, 500, 900, and 1,000 ng/mL as free base for morphine-3ß-glucuronide and 2.00, 5.00, 10.0, 20.0, 50.0, 100, 180 and 200 ng/mL as free base for morphine-6ß-glucuronide.

Five pools of quality control (QC) samples were prepared in human potassium EDTA plasma at concentrations of 1.00 ng/mL (lower limit of quantitation, LLQ), 3.00 ng/mL (QC-Low), 40.0 ng/mL (QC-Middle), 80.0 ng/mL (QC-High) and 400 ng/mL (QC-diluted) for hydromorphone and morphine, 0.200 ng/mL (LLQ), 0.600 ng/mL (QC-Low), 8.00 ng/mL (QC-Middle), 16.0 ng/mL (QC-High) and 80.0 ng/mL (QC-diluted) for norfentanyl, and 0.100 ng/mL (LLQ), 0.300 ng/mL (QC-Low), 4.00 ng/mL (QC-Middle), 8.00 ng/mL (QC-High) and 40.0 ng/mL (QC-diluted) for fentanyl. QC-diluted was processed after a 20-fold dilution in blank human potassium EDTA plasma. For morphine-3ß-glucuronide and morphine-6ß-glucuronide five pools of QC samples were prepared in human potassium EDTA plasma at concentrations of 10.0 ng/mL (LLQ), 30.0 ng/mL (QC-Low), 400 ng/mL (QC-Middle), 800 ng/mL (QC-High) and 4,000 ng/mL (QC-diluted) for morphine-3ß-glucuronide and 2.00 ng/mL (LLQ), 6.00 ng/mL (QC-Low), 80.0 ng/mL (QC-Middle), 160 ng/mL (QC-High) and 400 ng/mL (QC-diluted) for morphine-6ß-glucuronide. Pools of QC samples were aliquotted and stored at T<-70°C upon processing.

1.3 Plasma sample preparation for hydromorphone, morphine, fentanyl and norfentanyl

Aliquots of 200 µL of plasma samples were transferred into 1.5-mL microcentrifuge tubes, and 100 µL of internal standard solution and 100-µL aliquots of acetone were added.

Hereafter, the samples were vigorously mixed for 5 minutes and then centrifuged at 18,000 x *g* at ambient temperature for 10 minutes. The supernatant was transferred into 2-mL microcentrifuge tubes after which 100 µL of 4% ammonium hydroxide solution and 1-mL ethyl acetate was added. Hereafter, the samples were vigorously mixed for 5 minutes and then centrifuged at 18,000 x *g* at ambient temperature for 10 minutes. The organic phases were transferred into 4.5-mL glass tubes and evaporated under a stream of nitrogen at T=70°C. The residues were resuspended in 100-µL aliquots of methanol/water/formic acid (10:90:0.1, v/v/v) by ultrasonification for 30 seconds. After centrifugation of 2 minutes at 4,000 x *g*, the supernatants were transferred into 350-µL 96-well plates, which were placed into a chilled (T=10°C) autosampler, from which aliquots of 10 µL were injected onto the HPLC column.

1.4 Plasma sample preparation for morphine-3ß-glucuronide and morphine-6ß-glucuronide

Aliquots of 850 µL of 10 mM ammonium carbonate pH 8.8 were added to 100 µL plasma samples in 1.5-mL microcentrifuge tubes. Hereafter 50 µL internal standard solution was added. The SPE C18 cartridges (Waters, Etten-Leur, The Netherlands) were pre-washed with 1-mL methanol followed by 1-mL water and 1-mL of a 10 mM ammonium carbonate pH 8.8 solution. Hereafter 1-mL of plasma samples were loaded on the SPE C18 cartridges. Subsequently the cartridges were washed with 1-mL 10 mM ammonium carbonate at pH 8.8 after which the analytes were eluted with 0.5 mL methanol and evaporated under a slightly stream of nitrogen at T=70°C. The residues were resuspended in 100-µL aliquots of acetonitrile/water/formic acid (3:97:0.1, v/v/v) by ultrasonification. After centrifugation of 2 minutes at 4,000 x *g*, the supernatants were transferred into 350-µL 96-well plates, which were placed into a chilled (T=10°C) autosampler, from which aliquots of 10 µL were injected onto the HPLC column.

1.5 Equipment

The UPLC-MS/MS system from Waters Chromatography B.V. (Etten-Leur, The Netherlands) consisted of a Waters Aquity UPLC Sample Manager, coupled to a Waters TQ Detector. QuanLynx, an Application Manager included with MassLynx V4.1 SCN627 software package, was used for the acquisition and processing of data.

1.5.1 Chromatographic conditions

Hydromorphone, morphine, fentanyl and norfentanyl were separated on an Aquity UPLC® BEH C18 column 1.7 µm, 100 mm x 2.1 mm, (Waters, Etten-Leur, The Netherlands) thermostatted at T=50°C. A gradient, at a flow-rate of 0.350 mL/min, was achieved with mobile phase A, composed of water/ammonium formate (0.02 mM), acidified with 0.1% formic acid and mobile phase B, composed of methanol acidified with 0.1% formic acid. Following a full loop injection of 10 µL, a linear gradient separation was used, with 10% to 100% of mobile phase B from 0.5 to 2 minutes, holding for 2 minutes with 100% mobile phase B and then 100% to 10% of mobile phase B over 0.1 minute, holding for 1.9 minutes for initial conditioning. The overall run time of the assay was 6 minutes. The needle wash solvent was composed of acetonitrile/methanol/water/2-propanol/formic acid (25:25:25:25:0.1, v/v/v/v/v). The column effluent was passed through the mass spectrometer and monitored. Morphine-3ß-glucuronide and morphine-6ß-glucuronide were separated on an VisionHT C18-P; 3µm 2.1x50 mm, (Grace, Breda, The Netherlands) thermostatted at T=40°C. A gradient, at a flow-rate of 0.250 mL/min, was achieved with mobile phase A, composed of water/ammonium formate (0.02 mM), acidified with 0.1% formic acid and mobile phase B, composed of acetonitrile acidified with 0.1% formic acid. A linear gradient separation was used, with 3% to 95% of mobile phase B from 3 to 3.1 minutes at a flow rate of 0.250 mL/min, holding for 3 minutes with 95% mobile phase B at a flow rate of 0.350 mL/min and then 95% to 3% of mobile phase B over 0.1 minute, holding for 3.9 minutes at a flow rate of 0.250 mL/min for initial conditioning. The overall run time of the assay was 10 minutes. The needle wash solvent was composed of acetonitrile/methanol/water/2-propanol/formic acid (25:25:25:25:0.1, v/v/v/v/v). The column effluent was passed through the mass spectrometer and monitored.

1.5.2 Mass spectrometry

Tandem mass spectrometry was performed in the positive ion electrospray ionization mode. Mass transitions of *m/z* were optimized for all analytes and there labeled internal standards by infusion of the respective analytes in acetonitrile/water/0.1% formic acid (40:60:0.1, v/v/v) via combined infusion. Optimal MS settings were manually adjusted. The desolvation gas was set at 800 mL/hour (nitrogen). The ionspray voltage was kept at 0.6 kV for morphine, hydromorphone, fentanyl and norfentanyl and their labeled internal standards and 0.5 kV for morphine-3ß-glucuronide and morphine-6ß-glucuronide and their labeled internal standards, with a source temperature of T=120°C and desolvation temperature of T=350°C. The dwell times were set at 50 ms and the inter-channel delay at 100 ms. Multiple reaction monitoring (MRM) mode was applied for the quantitation and summarized in Table 1. The primary ion transition was used as quantifier while the primary to secondary ion ratios were used to show the quality of the observed peaks. See Table 1 for the secondary daughter ions analysed. The collision cell pirani pressure was set at ~$5e^{-3}$ mbar (argon).

1.5.3 Quantitation

Calibration curves were generated using peak area ratios of analytes to internal standards versus the known concentrations with a linear regression equation of 1/concentration^2.

1.6 Method validation

The quantitative UPLC-MS/MS method was validated in accordance with the Guidance for Industry, Bioanalytical Method Validation, as specified by the FDA (http://www.fda.gov/CDER/guidance/4252fnl.htm).

Potential presence of endogenous contaminating compounds that may interfere with the analytical assay was determined by analyzing blank human potassium EDTA plasma samples of ten different lots. The following clinical co-administered drugs were investigated for interference with the analytical method: dexamethasone, domperidone (Motilium®), lactulose (Legendal®), lorazepam (Temesta®), oxazepam, paracetamol; metoclopramide (Primperan®), granisetron (Kytril®), ondansetron (Zofran®), ranitidine (Zantac®) and aprepitant (Emend®). All drugs have been dissolved/diluted in water to a final concentration of 1 mg/mL followed by a 1,000 fold dilution in blank human potassium EDTA plasma for investigation of interference with morphine, hydromorphone, fentanyl and norfentanyl and a 200 fold dilution in blank potassium EDTA plasma for investigation of interference with morphine-3ß-glucuronide and morphine-6ß-glucuronide. Aliquots were subsequently diluted in plasma containing the different drugs to a final concentration of 5.00 ng/mL for morphine, hydromorphone, fentanyl and norfentanyl and 250 ng/mL for morphine-3ß-glucuronide and morphine-6ß-glucuronide, which haves been processed and compared to equal concentrations in blank human potassium EDTA plasma.

Accuracy (ACC), within-run precision (WRP) and the between-run precision (BRP) were determined, for both calibration curve ranges, by analyzing 5 replicates of pools of LLQ and QC samples independently over a three-run (four-run for QC-Diluted) period, with the calibration curve standards processed in duplicate. The ACC, WRP and BRP at the level of the LLQ and QC samples were calculated by one-way analysis of variance, using the run as the variable.

For the validation of the LLQ, besides the validation of the pools as described above, blank human potassium EDTA plasma of 10 different volunteers were spiked at concentrations of 1.00 ng/mL for morphine and hydromorphone, 0.200 ng/mL for norfentanyl, 0.100 ng/mL for fentanyl, 2.00 ng/mL for morphine-6ß-glucuronide and 10.0 ng/mL for morphine-3ß-glucuronide and quantitated in a separate run.

Carry-over was evaluated in the validation runs by injection of a double blank processed sample directly after the highest calibration standard. The response for the analytes should be <20% of the response at the LLQ and <5% of the response of the internal standards in the calibration standards.

Extraction recovery (RE) was determined by comparing the MS/MS response of morphine, hydromorphone, fentanyl, norfentanyl and morphine-3ß-glucuronide and morphine-6ß-glucuronide at 40.0 ng/mL for morphine and hydromorphone, 4.00 ng/mL for fentanyl, 8.00 ng/mL for norfentanyl and 100 ng/mL and 20.0 ng/mL for morphine-3ß-glucuronide and morphine-6ß-glucuronide respectively, spiked in triplicate into six different lots of blank potassium EDTA plasma before extraction, to the MS/MS responses of the analytes spiked in triplicate into extracts of blank human potassium EDTA plasma after extraction, corrected for the evaporated volume of organic phase. Matrix effect (ME) was determined by comparing the MS/MS response of neat standard solution compared to MS/MS response of six different lots of blank matrices, supplemented with the same amount of standards as used for the neat standard solutions, but added after extraction.

The stability at ambient temperature of morphine, hydromorphone, fentanyl, norfentanyl, morphine-3ß-glucuronide and morphine-6ß-glucuronide in human potassium EDTA plasma was tested with QC-Low and QC-High and QC-Diluted for a period of 18 hours. Stability was tested after 3 freeze-thaw cycles, in which the samples were thawed for at least 30 minutes followed by refreezing for at least 18 hours. Long-term stability at T< -70°C in human potassium EDTA plasma has been investigated for 39 months for morphine-3ß-glucuronide and morphine-6ß-glucuronide, and 76 months for morfine, hydromorphone,fentanyl and norfentanyl using the same QC samples as described above. Autosampler stability was tested in triplicate at the concentration of QC-Low and QC-High. The QC samples were processed and repeatedly injected on different time points.

2 Results and discussion

2.1 LC-MS/MS conditions and method development

To determine the most abundant product ions for hydromorphone, morphine, fentanyl, norfentanyl, morphine-3ß-glucuronide and morphine-6ß-glucuronide, MS/MS experiments were carried out by tuning the compounds with combined infusion. After optimization of the mass spectrometric parameters, the column effluent was monitored using the multiple reaction monitoring mode. The selected product ions, the optimal cone voltages and collision energies are presented in Table 1.

Several pre-treatment procedures which lead to purified extracts are available, including protein precipitation, solid-phase and liquid-liquid extraction. For bioanalysis of opioid concentrations in plasma of patients, we needed lower LOQ values than could be reached with protein precipitation alone (18).

For the quantification of morphine, hydromorphone, fentanyl and norfentanyl, a liquid-liquid extraction procedure was applied after a deproteinization step with acetone. Optimal extraction recoveries were achieved with ethyl acetate as extractor and alkalised plasma with ammonium hydroxide after the deproteinization step with acetone. Since morphine glucuronides are hydrophilic compounds, a more laborious and time-consuming solid phase extraction with ammonium carbonate buffer pH 8.8 was needed for pre-treatment of plasma samples containing morphine-3ß-glucuronide and morphine-6ß-glucuronide.

Table 1 MS/MS settings.

Analyte	Scan window	Parent	Product ion	Product ion	Collision
			Prim.	Sec.	
	(minutes)	(m/z)	(m/z)	(m/z)	(V)
Morphine	0.8-2.00	286	201	268	26
Morphine-d6	0.8-2.00	292	201	229	26
M3G	0.8-2.00	462	286	268	32
M3G-d3	0.8-2.00	465	289	271	29
M6G	0.8-2.00	462	286	268	34
M6G-d3	0.8-2.00	465	289	229	32
Hydromorphone	0.8-2.00	286	185	227	30
Hydromorphone-d6	0.8-2.00	292	185	227	30
Fentanyl	1.80-3.10	337	188	134	28
Fentanyl-d5	1.80-3.10	342	188	137	33
Norfentanyl	1.80-3.10	233	177	150	20
Norfentanyl-d5	1.80-3.10	238	182	155	20

Analysis times were relatively short by using a linear gradient with 6 minutes for the analysis of morphine, hydromorphone, fentanyl and norfentanyl and 10 minutes for the analysis of morphine-3ß-glucuronide and morphine-6ß-glucuronide. Because of the same molecular mass and therefore the necessarily baseline separation a C18 polar endcapped column was used. This enables the use of a high aqueous mobile phase at the start of the gradient.

2.2 Assay performance

The observed peak area ratios using a weighting factor of $1/(\text{concentration})^2$ were linear ($r \geq 0.9969$) in the concentration range of 1.00 to 100 ng/mL for morphine and hydromorphone and 0.100 to 10.0 ng/mL for fentanyl and 0.200 – 20.0 ng/mL for norfentanyl and 10.0 – 1,000 ng/ml for morphine-3ß-glucuronide and 2.00 – 200 ng/mL for morphine-6ß-glucuronide in human potassium EDTA. No interference was found with any of the opioids or the deuterated internal standards in all of the ten

blank plasma samples. Interference from potentially co-administered drugs was seen with high concentrations (>1 µg/mL) of domperidone on the signals of fentanyl and norfentanyl. As stable labeled internal standards were used, this had no impact on the quantitation in case the peak areas were equal or higher than the peak areas at the LLQ. In patients the Cmax (23-80 ng/mL) of high dose domperidone is far below 1 µg/mL, and has therefore no clinical relevant impact on the quantitation of fentanyl or norfentanyl (19).

The LLQ was validated at 1.00 ng/mL for hydromorphone and morphine, at 0.200 ng/mL for norfentanyl, at 0.100 ng/mL for fentanyl, at 10.0 ng/mL for morphine-3ß-glucuronide, and at 2.00 ng/mL for morphine-6ß-glucuronide. The LLQ samples were prepared in 10 different plasma lots and analyzed in the first validation run. Subsequently, the tested LLQ samples were pooled and used as a QC sample for the three following validation runs. For morphine, the measured concentrations for all of the independently spiked plasma samples fell within the acceptable range of accuracy of 80 - 120%, with an average concentration of 0.985 ± 0.120 ng/mL. The measured concentrations of hydromorphone for all 10 independent potassium EDTA plasma samples fell within the acceptable range of accuracy, with an average observed concentration of 1.02 ± 0.0840 ng/mL. The average concentration for fentanyl for all 10 independent potassium EDTA plasma samples fell within the acceptable range of accuracy, with an average observed concentration of 0.0913 ± 0.00526 ng/mL. For norfentanyl, measured concentrations in 8 of 10 independent samples fell within the acceptable range of accuracy, with an average concentration of 0.214 ± 0.0313 ng/mL For morphine-3ß-glucuronide and morphine-6ß-glucuronide all measured concentrations for all independent potassium EDTA plasma samples fell within the acceptable range of accuracy, with average observed concentrations of 10.1 ± 0.770 and 2.13 ± 0.282 ng/mL respectively. The within-run and between-run precisions and the accuracies at five tested concentrations, including at the level of the LLQ, are summarized in Table 2.

The carry-over test was found to be acceptable for all compounds, see Table 3. The response for all analytes was less than 20% of the response at the LLQ and less than 5% of the response of the internal standards.

The recovery and matrix effect of the opioids has been determined in six different lots of human potassium EDTA plasma. The mean measured extraction efficiencies for morphine, hydromorphone, fentanyl and norfentanyl were 67%, 60%, 85% and 72%, respectively, and 82% and 121% for morphine-3ß-glucuronide and morphine-6ß-glucuronide, respectively. A slight enhancement of matrix effect was observed for morphine, hydromorphone and morphine-6ß-glucuronide, which is not an uncommon phenomenon, but has no effect on the quantification because the used deuterated internal standard compensate for the matrix effect. See Table 3 for the results of recovery and matrix factor.

Table 2 Calculations of the between-run and within-run precisions and the average accuracy of the LLQ and QC samples[1]

Sample	Spiked (ng/mL)	GM (ng/mL)	ACC (%)	WRP (%)	BRP (%)	n[3]
Morphine						
LLQ	1.00	0.919	91.9	10.3	6.71	13 of 15
Low	3.00	2.88	96.0	7.92	8.67	14 of 15
Middle	40.0	38.1	95.3	3.60	4.50	15 of 15
High	80.0	77.5	96.9	2.23	1.76	15 of 15
Diluted	400	369	92.3	4.50	3.64	20 of 20
Morphine-3ß-glucuronide						
LLQ	10.0	8.40	84.0	5.16	#[2]	13 of 15
Low	30.0	26.2	87.3	3.46	0.862	12 of 15
Middle	400	378	94.5	3.82	1.68	15 of 15
High	800	772	96.5	2.89	2.18	15 of 15
Diluted	4	3,824	95.6	1.79	#[2]	15 of 15
Morphine-6ß-glucuronide						
LLQ	2.00	1.74	87.0	16.2	9.12	12 of 15
Low	6.00	5.84	97.3	10.2	5.61	12 of 15
Middle	80.0	77.1	96.4	5.38	#[2]	15 of 15
High	160	156	97.5	4.16	#[2]	15 of 15
Diluted	400	422	105.5	5.16	#[2]	14 of 15
Hydromorphone						
LLQ	1.00	0.862	86.2	6.58	#[2]	13 of 15
Low	3.00	2.80	93.3	4.09	#[2]	15 of 15
Middle	40.0	39.0	97.5	4.35	1.85	15 of 15
High	80.0	77.9	97.4	1.87	#[2]	15 of 15
Diluted	400	364	91.0	3.23	5.58	17 of 20
Fentanyl						
LLQ	0.100	0.0888	88.8	5.52	6.12	14 of 15
Low	0.300	0.269	89.7	4.29	1.07	13 of 15
Middle	4.00	3.76	94.0	7.01	#[2]	15 of 15
High	8.00	7.40	92.5	1.37	1.86	15 of 15
Diluted	40.0	35.4	88.5	2.91	4.15	17 of 20
Norfentanyl						
LLQ	0.200	0.199	99.5	16.3	14.6	13 of 15
Low	0.600	0.604	100.7	7.82	0.406	15 of 15
Middle	8.00	7.75	96.9	4.64	#[2]	15 of 15
High	16.0	15.3	95.6	3.86	#[2]	15 of 15
Diluted	80.0	73.9	92.4	3.64	#[2]	20 of 20

Abbreviations: GM, grand mean; WRP, within-run precision; BRP, between-run precision; ACC, average accuracy;
[1], n=5 in 3 separate runs (4 runs at the QC Diluted for morphine, hydromorphone, fentanyl and norfentanyl)
[2], no additional variation observed by performing the assay in different runs.
[3], number of individual samples falling within acceptable range of accuracy of 85-115% (80-120% at LLQ)

Table 3 Calculations of carry-over, recovery and matrix factor

	Carry-over (%)	REC (%)	CV (%)	Matrix factor	CV (%)
Morphine	5.5	67.4	6.8	1.43	12.1
Hydromorphone	10.5	60.5	8.5	1.47	15.3
Fentanyl	4.8	85.4	3.0	1.04	3.3
Norfentanyl	0.28	72.4	9.5	1.21	13.3
M3G	0.53	82.5	1.9	1.21	16.3
M6G	2.7	121	18.2	1.54	4.5

The stability of morphine, hydromorphone, fentanyl and norfentanyl and the metabolites morphine-3ß-glucuronide and morphine-6ß-glucuronide was tested in triplicate at the concentrations of QC Low, QC-High and QC-Diluted. QC-samples were incubated for 18 hours at ambient temperature and stability was tested after three freeze-thaw cycles. All tested drugs showed to be stable under these circumstances.

Hydromorphone, morphine, fentanyl and norfentanyl were stable in potassium EDTA plasma for at least 76 months when stored at T<-70°C. Morphine-3ß-glucuronide and morphine-6ß-glucuronide were stable for at least 39 months when stored at T<-70°C (Table 4). All compounds showed to be stable as processed sample in the autosampler for at least 17 hours.

The described analytical methods were applied to clinical studies to investigate the pharmacokinetics of opioids. A representative concentration-time curve of a cancer patient receiving several opiods is presented in figure 2.

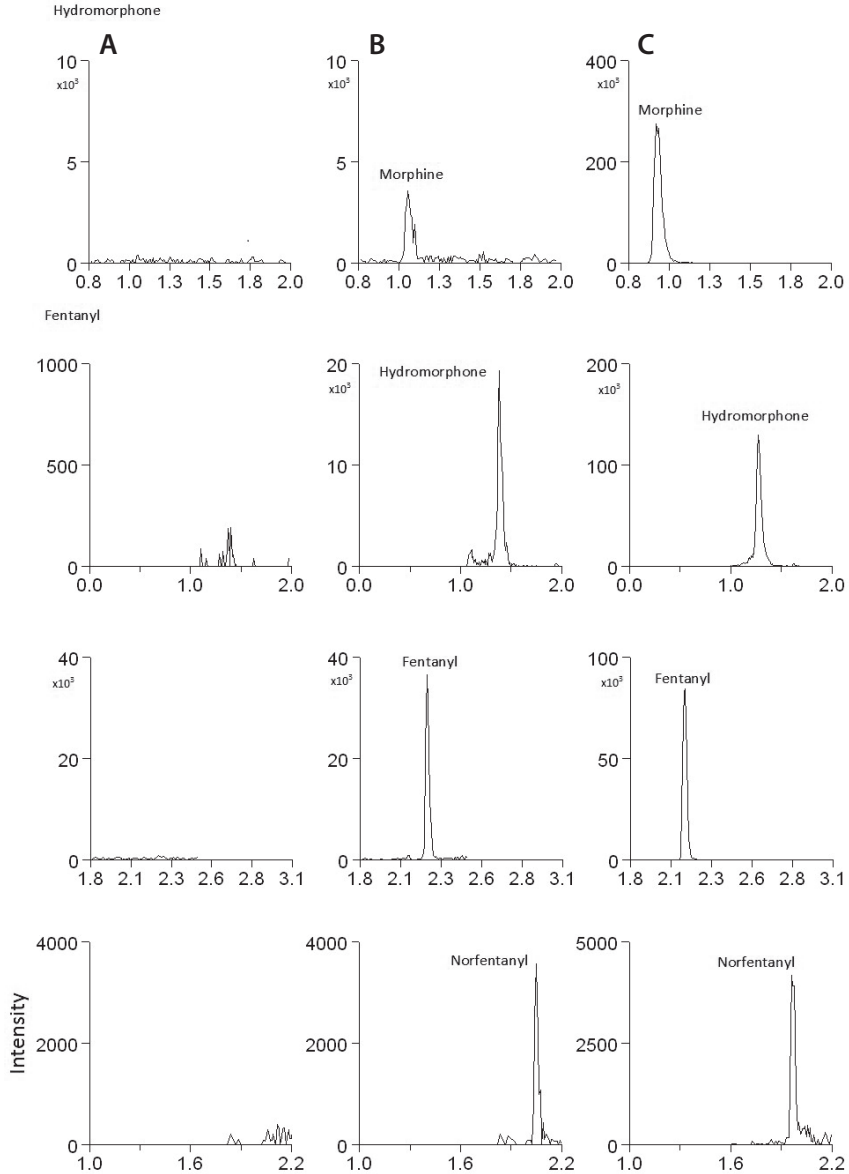

Figure 1 Representative chromatograms of a double blank processed plasma sample (**A**), a plasma sample spiked at the concentration of the LLQ (**B**) and a plasma sample collected after opioid administration 65.9 ng/mL morphine, 6.50 ng/mL hydromorphone, 0.466 ng/mL fentanyl, 0.603 ng/mL norfentanyl and 1510 ng/mL morphine-3ß-glucuronide and 240 ng/mL morphine-6ß-glucuronide after 5-fold dilution in blank human plasma (**C**).

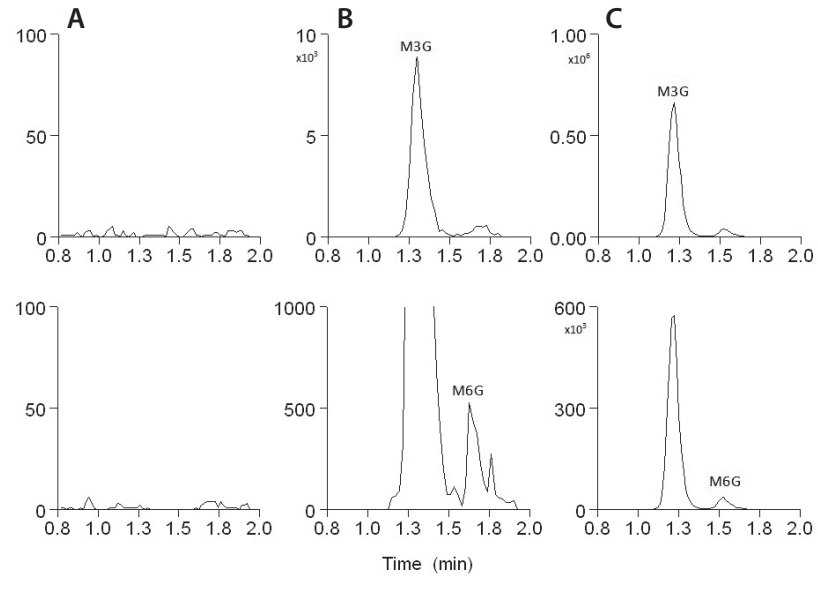

Figure 1 Continued.

Table 4 Stability

Condition	Plasma (% to control)		
	QC Low	QC High	QC Diluted
Morphine			
Ambient temp (18h)	107	95.7	107
3 freeze/thaw cycles	98.1	97.3	102
Processed sample (T=10°C)	94.6	115	ND[1]
T<-70°C (76 months)	103	97.0	ND[1]
Hydromorphone			
Ambient temp (18h)	100	97.9	108
3 freeze/thaw cycles	102	103	96.2
Processed sample (T=10°C)	103	101	ND[1]
T<-70°C (76 months)	103	114	ND[1]
Fentanyl			
Ambient temp (18h)	109	104	110
3 freeze/thaw cycles	110	106	102
Processed sample (T=10°C)	97.6	95.3	ND[1]
T<-70°C (76 months)	104	96.9	ND[1]
Norfentanyl			
Ambient temp (18h)	96.0	102	106
3 freeze/thaw cycles	93.8	102	96.3
Processed sample (T=10°C)	99.2	111	ND[1]
T<-70°C (76 months)	90.4	92.8	ND[1]
Morphine-3ß-glucuronide			
Ambient temp (18h)	95.9	99.8	103
3 freeze/thaw cycles	96.2	98.7	106
Processed sample (T=10°C)	99.8	99.4	ND[1]
T<-70°C (39 months)	108	113	113
Morphine-6ß-glucuronide			
Ambient temp (18h)	97.2	98.3	98.8
3 freeze/thaw cycles	97.8	99.6	99.5
Processed sample (T=10°C)	96.8	108	ND[1]
T<-70°C (39 months)	108	108	102

[1], Not Determined

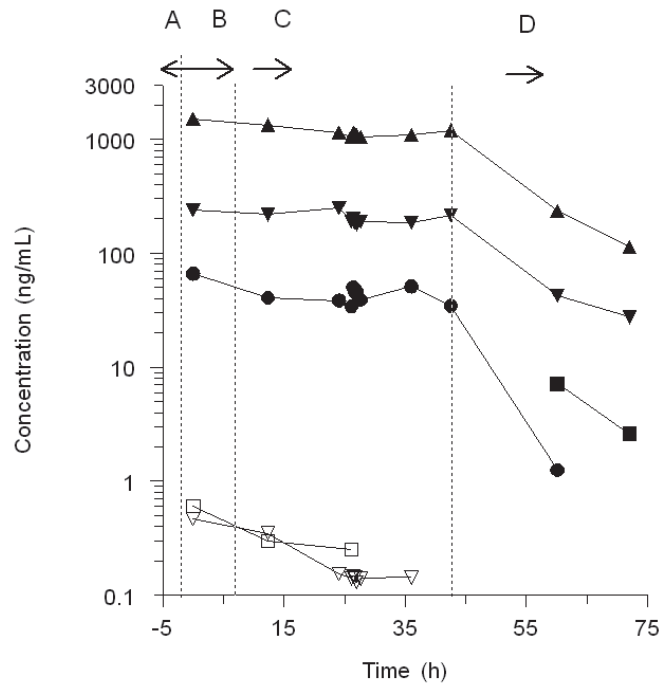

Figure 2 Plasma concentration-time profile of fentanyl (-△-), norfentanyl (-□-), morphine (-●-), M3G (-▲-), M6G (-**+**) and hydromorphone (-◇-) in a patient who was treated for severe pain with 250 µg/h fentanyl during period **A**, 12 mg/h morphine during period **B** 6 mg/h morphine during period **C** and 1.6 mg/h hydromorphone during period **D**.

3 Conclusion

Selective, sensitive, accurate and precise methods have been validated for hydromorphone, morphine, fentanyl, norfentanyl and the metabolites morphine-3ß-glucuronide and morphine-6ß-glucuronide in human potassium EDTA plasma, which meets the current requirements of bioanalytical method validation. Compared to other described methods, small volumes of plasma are used and the method described here is much more sensitive for fentanyl and norfentanyl compared to other published methods and therefore suitable for pharmacokinetic studies. Also for the detection of morphine, hydromorphone, fentanyl and norfentanyl, a less time-consuming liquid-liquid extraction is used.

The methods are currently successfully applied for the bioanalysis of opioid concentrations in plasma of cancer patients participating in clinical studies in which opioids are administered for the treatment of moderate to severe cancer-related pain.

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4

Effects of smoking and body mass index on the exposure of fentanyl in patients with cancer

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Abstract

The transdermal fentanyl patch is widely used to treat cancer-related pain despite its wide inter- and intrapatient variability in pharmacokinetics. The aim of this study was to investigate whether smoking and body size (i.e. body mass index) influence fentanyl exposure in patients with cancer. These are factors that typically change during treatment and disease trajectories. We performed an explorative cohort study in patients with cancer using transdermal fentanyl patches (Durogesic®), by taking a blood sample for pharmacokinetic analysis one day after applying a patch in patients with a stable fentanyl dose. A total of 88 patients were evaluable. Although no statistically significant difference was found, the plasma concentrations of non-smokers was 28% (95% CI [-14%; +89-%]) higher than those of smokers normalizing for a dose of 25µg/min. Patients with a low BMI (< 20 kg/m2) had almost similar (10% (95% CI [-39%; +97%]) higher) plasma concentrations compared to patients with a high BMI (> 25 kg/m2). A wider variation in fentanyl plasma concentrations was found in this study than anticipated. Due to this variation, studies in larger patient cohorts are needed to further investigate the effect of smoking on plasma concentration of fentanyl and thereby clarify the clinical significance of our findings.

Introduction

Fentanyl is one of the most commonly used (strong acting) opioids to treat cancer-related pain [1-3] and it is often preferred over morphine, especially in patients with renal failure. Additionally, fentanyl usually results in less obstipation than other opioids [4-6]. The fentanyl transdermal patch has been used since decades to treat chronic pain. Fentanyl is also available in a liquid formulation for intravenous and subcutaneous administration and in various immediate release forms for oromucosal and nasal use [7, 8]. Fentanyl is highly lipophilic and is therefore rapidly absorbed by the subcutaneous fat-tissue. After placement of the patch fentanyl is absorbed by the skin. When the patch is removed systemic fentanyl concentration will slowly decrease as a result of fentanyl release from subcutaneous depots (formed below the patch) [7, 9, 10].

Fentanyl is mainly metabolized in the liver by cytochrome P450 (CYP) enzymes, primarily by the CYP3A4 isozyme into the pharmacologically inactive metabolite norfentanyl by N-dealkylation. Fentanyl is mostly excreted by the kidney of which approximately 10% is unchanged. A minor part is excreted through the feces. There is a wide inter- and intra-patient variability in fentanyl pharmacokinetics (PK). Several factors have been studied to clarify this variability in fentanyl pharmacokinetics; liver function, strong CYP3A4 inhibitors and inducers and adding local heat to transdermal patches evidently affect fentanyl pharmacokinetics but only partly explain the wide variability mentioned [7]. Other unknown factors may also be contributing to this variation.

Two patient characteristics that may change during various stages of cancer are body size measures and smoking habits, while their effects on fentanyl exposure in patients with cancer are currently unknown. The body mass index (BMI) is one of the most commonly used body size measures and is calculated by weight (in kilogram) divided by the square of height. In patients with advanced disease a decrease in BMI is common and this may lead to a changed body composition, changed thickness of the skin, and changed skin permeability. Some studies already studied the influence of BMI on fentanyl pharmacokinetics with contradictory results. One study reported that cachectic patients with cancer treated with transdermal fentanyl patches had significantly lower fentanyl plasma concentrations compared to normal weight patients [11]. Other studies did not find differences in fentanyl levels between normal weight and low weight patients with cancer [12, 13].

Smoking is a daily routine for many patients with cancer. If smoking alters fentanyl exposure, a change in smoking habits like (re-)starting or quitting smoking may influence fentanyl pharmacokinetics and therefore its effects on pain. The polycyclic aromatic hydrocarbons in cigarette smoke are believed to be responsible for the induction of CYP iso-enzymes [14, 15]. Cigarette smoking was shown to induce drug metabolism in patients using diazepam, haloperidol and/or caffeine [16-18].

Furthermore, smoking patients showed a lower exposure to erlotinib and irinotecan compared to non-smoking patients. Like fentanyl, erlotinib and irinotecan are also partly metabolized by CYP3A4 [19, 20]. Therefore, we hypothesized that smoking may have an effect on the exposure of fentanyl.

The aim of this prospective cohort study was to investigate the influence of BMI and smoking on the exposure of fentanyl in patients with cancer using a fentanyl patch.

Methods

Our study was performed as a prospective single-center pharmacokinetic study at the Erasmus MC Cancer Institute. Patients were included from 1st of April 2014 until 27th of October 2015. Inclusion criteria were: patients with cancer ≥ 18 years, using a stable dose of a transdermal fentanyl (Durogesic®) for at least 8 days irrespective of the dose used and given written informed consent. Exclusion criteria were: use of fentanyl rescue medication (other opioids were allowed), the use of strong CYP inhibitors or inducers and serious psychiatric illness, confusion or intellectual disability. Smoking was defined as smoking tobacco daily. Non-smoking patients were defined as patients who had never smoked, or quit smoking at least one month before PK sampling. Patients were divided in three BMI groups; BMI <20 kg/m^2 (low), BMI 20-25 kg/m^2 (normal) and BMI >25 kg/m^2 (high). Patients applied the patch to the upper arm. One venous blood sample was taken from the contralateral arm approximately 24 hours after application of a new patch. The blood samples were collected in potassium EDTA coated tubes. Patients were not evaluable when the appropriate blood samples were not taken. Lab results of levels of creatinine, estimated glomerular filtration rate (eGRF), calculated by Modification of Diet in Renal Disease (MDRD); [formula: eGFR (ml/min/1.73 m^2) = 32788 x serum Creatinine (µmol/l) $^{-1.154}$ x age (years) $^{-0.203}$ x (0.742 when female) x (1.210 when of African descent)], aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, albumin and alkaline phosphatase (ALP) were assessed.

The study was approved by the medical ethics review board of the Erasmus Medical Center (MEC 13.412) on the 27th of March 2014 and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. The trial was registered in the Dutch Trial Register (Trial registration ID: NTR4672) in July 2014. The authors confirm that all ongoing and related trials for this drug/intervention are registered.

Measurements of fentanyl plasma concentrations

Fentanyl in plasma was quantitated using a validated UPLC-MS/MS method consisting of a Waters Acquity UPLC sample manager coupled to a triple quadruple mass spectrometer operating in the multiple reaction monitoring mode (MRM) with positive ion electrospray ionization (Waters, Etten-Leur, The Netherlands). The multiple reaction monitoring transition was set at 337→188.

Chromatographic separation of fentanyl was achieved on an Acquity UPLC® BEH C18 1.7 µm 2.1 x 100 mm column eluted at a flow-rate of 0.350 mL/min on a gradient of methanol. The overall cycle time of the method was 6 minutes. The calibration curves were linear over the range of 0.1 to 10 ng/mL with the lower limit of quantitation validated at 0.1 ng/mL for fentanyl. The within and between-run precisions at five tested concentrations, including the LLQ, were ≤ 5.5 % and ≤ 6.1 %, respectively, while the average accuracy ranged from 86.2 % to 97.5%. The extraction of 200 µL of plasma involved a deproteinization step with acetone followed by a simple liquid extraction with ethyl acetate. The organic phase was evaporated and subsequently dissolved in 100 µL methanolic solutions, from which aliquots of 10 µL were injected into the UPLC-MS/MS system.

Statistics

For both the analysis on the effect of smoking and of BMI, a difference in exposure of fentanyl of 25% was judged clinically relevant. The inter-patient relative standard deviation in fentanyl pharmacokinetics was assumed to be 25%. Because two primary questions were to be answered, the Bonferroni correction was applied to account for multiple testing, resulting in a two-sided alpha of 0.025. Given a power of 80%, at least 20 patients were required in each BMI group (low versus high) to detect a difference based on the Student's t-test. The assumption was that 25% of our population smoked. To include 20 smokers approximately 80 patients were needed in total. To compensate for non-evaluable patients (due to missing samples or other potential reasons) we aimed to include 100 patients.

As fentanyl has linear PK in the dose range used, doses were normalised to a dose of 25 µg/h for comparisons between patients [21, 22]. As the normalized plasma fentanyl concentrations turned out to follow a log-normal distribution, analyses were performed on the log-transformed concentrations and therefore, results will be presented as geometric means. These take into account the skewness of the data in contrast to arithmetic means which would have given unrealistic summary measures of the data. T-tests were performed on log-transformed data to compare 2 groups; smokers to non-smokers and low-BMI to high-BMI patients with respect to baseline characteristics. Bonferroni-corrected 95% confidence intervals were obtained by constructing 97.5% confidence intervals since the correction means that instead of alpha=0.05 an alpha of 0.025 needs to be used.

Results

In total, 104 patients were included. Eighty-eight patients (39 males (44%) and 49 females (56%)) with a median age of 59.5 years (interquartile range (IQR) 53.5 – 67.0) completed the study and were evaluable, Fig 1. The demographic data of these evaluable patients are presented in Table 1. Twenty-seven patients (30.7%) were defined as smokers and 61 patients (69.3%) as non-smokers. In total, 20 patients had a BMI < 20 kg/m^2 (22.7%), 41 patients had a BMI between 20-25 kg/m^2 (46.6%) and 27 patients had a BMI > 25 kg/m^2(30.7%). Creatinine, eGFR, AST, ALT, bilirubin, albumin and ALP turned out to follow a log-normal distribution. Only AST levels were significantly lower in smokers compared to non-smokers (p=0.026), although this is unlikely to be clinically relevant . All patients had normal or limited (CTCAE grade 1) toxicities of creatinine, eGFR, AST, ALT, bilirubin, albumin or ALP.

Figure 1 Consort flow diagram.

Table 1 Patient characteristics

Variable	Total N=88	Smokers N=27	Non smokers N=61	BMI < 20 Median 18.5 N=20	BMI 20-25 Median 23.0 N=41	BMI > 25 Median 28.7 N=27
Sex, n(%)						
- Male	39 (44%)	15 (56)	24 (39)	13 (65)	16 (39)	10 (37)
- Female	49 (56%)	12 (44)	37 (61)	7 (35)	25 (61)	17 (63)
Age, years median and IQR	60 (53-67)	59 (54-66)	60 (53-68)	60 (49-68)	59 (55-65)	62 (53-69)
Height, cm median and IQR	170 (163-178)	173 (165-181)	169 (163-177)	175 (169-184)	170 (163-178)	167 (163-177)
Weight, kg median and IQR	66 (60-78)	69 (62-78)	65 (59-78)	54 (51-63)	64 (61-73)	81 (74-96)
Smoking (n, %)	27 (31)	27 (100)	0 (0)	6 (30)	13 (32)	8 (30)
Laboratory results (median (IQR)						
Creatinine[1]	66 (55-87)	70 (51-93)	65 (55-87)	59 (45-84)	62 (56-85)	78 (63-91)
MDRD[2]	88 (65-90)	89 (65-90)	87 (63-90)	90 (85-90)	90 (66-90)	71 (61-90)
AST[3]	27 (20-42)	22 (17-31)	30 (22-47)	22 (17-32)	28 (22-50)	31 (19-44)
ALT[4]	23 (13-34)	23 (13-27)	23 (15-40)	18 (14-27)	23 (13-34)	24 (13-45)
Bilirubin[5]	6 (4-8)	5 (4-8)	6 (4-8)	4 (3-7)	6 (4-8)	7 (5-9)
Albumin[6]	39 (36-43)	38 (35-42)	39 (37-44)	38 (32-42)	39 (36-43)	40 (37-44)
ALP[7]	114 (87-179)	93 (84-165)	124 (90-192)	108 (89-148)	118 (86-214)	111 (83-183)
Fentanyl patch dose (µg/h) median(IQR)	25 (12-50)	25 (12-50)	25 (12-50)	37 (15-50)	37 (12-69)	25 (12-25)

Normal ranges: [1] (55-90 umol/L); [2] (> 60 mL/min/1,73 m^2); [3] (< 31 U/L) ;[4] (< 34 U/L); [5] (<17 umol/L); [6] (35 -50 g/L);[7] (< 98 U/L)

The normalized plasma concentrations of non-smokers and smokers were not statistically different (p=0.32). The geometric means of the normalized plasma concentrations were 0.48 ng/ml (95% CI [0.38; 0.61]) for smokers and 0.62 ng/ml (95% CI [0.50; 0.76]) for non- smokers. The normalized plasma concentrations of non-smokers were 27.7% higher than those of smokers (95% CI [-13.8%; +89.1%], Fig 2).

The normalized plasma concentrations of patients with a low or high BMI were also not statistically different (0.62 ng/ml (95% CI [0.41; 0.93]) vs 0.56 ng/ ml (95% CI [0.40; 0.79]), p=1.00). Plasma concentrations of the low and high BMI groups did also not differ from the normal BMI group (0.56 ng/ml (95% CI [0.46; 0.68])). The concentration was 9.7% higher in patients with a low BMI as compared to patients with a high BMI (95% CI [-38.8%; 96.9%]) Fig 3).

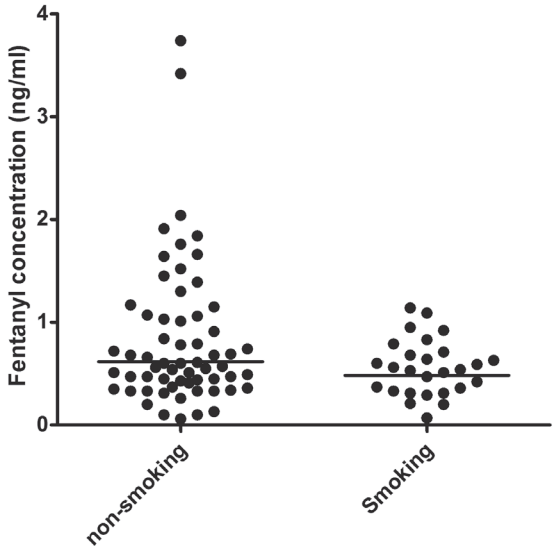

Figure 2 Normalized plasma fentanyl concentrations in patients; smokers and non-smokers. Horizontal line represents the median.

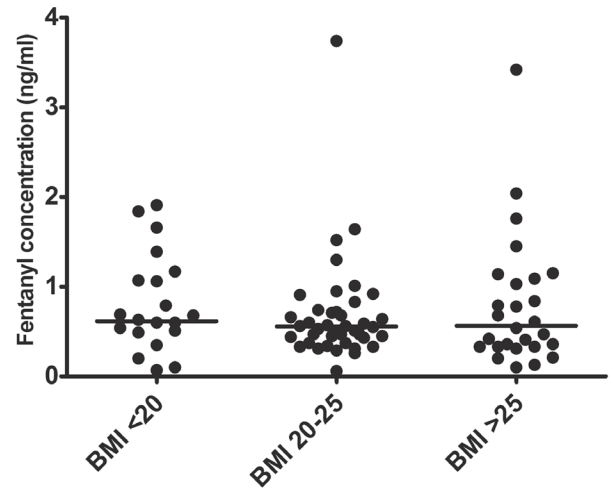

Figure 3 Normalized plasma fentanyl concentrations in patients; low, middle and high BMI patients. Horizontal line represents the median.

Discussion

Interestingly, in this study, the interindividual variation in plasma fentanyl levels (geometric coefficient of variation = 87%) was much larger than our original assumption. Consequently, our study was underpowered to find a statistically significant difference in fentanyl plasma concentration. Nonetheless, based on our findings, the 27.7% higher normalized plasma concentrations of non-smokers compared to smokers, we cannot exclude an effect of smoking on fentanyl exposure. Smoking is a factor that frequently changes during phases of disease in patients with cancer. Together with the hypothesis that smoking leads to induction of CYP3A4 [20, 23] and that fentanyl metabolism might also be influenced by other (unknown) metabolic pathways [24, 25] it might be interesting to study smoking in a larger cohort of patients with cancer. Nevertheless, other strong inducers like rifampicin [26] and carbamazepine or phenobarbital [24] had highly relevant inductive effects on fentanyl PK. The combination of rifampicin and oral transmucosal fentanyl citrate led to a significant lower exposure to fentanyl compared to fentanyl alone (2.20 vs 5.87 ng/mL). A population pharmacokinetic analysis showed a significantly higher fentanyl clearance when patients used CYP3A4 inducers compared to patients who did not use CYP3A4 inducers [24].

Unfortunately our study lacks information about the daily consumption of cigarettes and its use over the years. Probably, the amount of cigarettes smoked

daily influences the size of enzyme induction. All patients who were included in the non-smoking group did not smoke for at least one month before inclusion. The time to require maximum enzyme induction takes approximately two weeks [20, 23]. We therefore assume that potential late effects of enzyme induction by cigarette smoking were ruled out in this study since a minimum period of 4 weeks without smoking was required to be eligible for the non-smoking group.

Transdermal fentanyl is absorbed by the skin and a fentanyl depot concentrates in the upper skin layers. This is followed by uptake in the microcirculation and general circulation. Skin conditions of smokers are different compared to non-smokers; skin ageing is accelerated and leads to reduced functional capacity of the skin causing dryness of the skin and wrinkles [27-29]. This reduced skin condition might affect transdermal absorption of fentanyl. A limitation of this study is that we did not specify the skin conditions of the patients in this study and the sampling design did not allow us to describe the absorption phase. BMI did not significantly influence the exposure to transdermal fentanyl in our cohort of patients, which is in line with earlier studies [12, 13]. Hypoalbuminemia is common in patients with a low BMI and because of the lipophilic character of fentanyl, the binding of fentanyl to plasma proteins like albumin might be influenced by the plasma concentration of albumin [7, 12]. In our study, the albumin levels were within normal ranges and comparable in all BMI groups. The combination of low BMI and normal albumin did not influence fentanyl plasma concentration in another study [12]. Only a study by Heiskanen et al. showed significantly lower plasma fentanyl levels in cachectic patients with cancer compared to normal weight patients [11]. Probably this is due to the extremely low BMI group of < 16 in their study. An explanation for their finding might be lower skinfold thickness in cachectic patients. The thinner lipophilic part of the skin possibly impairs the absorption of lipophilic fentanyl [9]. Possibly, other factors, not studied, related to BMI and cachexia like CRP, skinfold and skin condition might also influence absorption.

In summary, we conducted a prospective cohort study to investigate the influence of smoking or BMI on plasma fentanyl concentrations. For both factors we did not find any evidence for an effect nor the lack of an effect. This study was underpowered because of unexpectedly large variations in fentanyl levels between patients. To study the effect of smoking and BMI on fentanyl levels a larger patient population needs to be tested.

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5

Influence of aprepitant and localization of the patch on fentanyl exposure in patients with cancer using transdermal fentanyl

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Abstract

Background & Objectives: The cutaneous fentanyl patch is widely used to treat continuous pain in patients with cancer. Its use is hampered by a high inter- and intrapatient pharmacokinetic variability. Factors that influence this pharmacokinetic variability are largely unclear. The aim of these studies was to test if common patient variables, i) the use of the moderate CYP3A4 inhibitor aprepitant and ii) the localization of the fentanyl patch (upper arm versus thorax) influence systemic exposure to fentanyl in patients with cancer using a transdermal fentanyl patch.

Methods: We performed two prospective cross-over pharmacokinetic intervention studies. Both studies had two eight-day study periods. At day 8 of each study period blood samples were collected for pharmacokinetic analysis. In each study 14 evaluable patients were included.

Results: The $AUC_{0\text{-}6h}$ of fentanyl was 7.1% (95% CI: -28% to+19%) lower if patients concurrently used aprepitant, compared to the period when patients used fentanyl only. The $AUC_{0\text{-}4h}$ of fentanyl was 7.4% (95% CI: -22% to +49%) higher when the cutaneous fentanyl patch was applied to the upper arm compared to application at the thorax.

Conclusion: Neither the concurrent use of aprepitant, nor the localization of the fentanyl patch showed a statistically significant influence on fentanyl pharmacokinetics.

1 Introduction

Since decades the fentanyl cutaneous patch is used to treat chronic cancer pain (1). The patch is widely used mainly because of its patient-friendly administration route (2, 3). This patch is applied to the skin and has to be changed every 72 hours/ 3 days. Fentanyl is absorbed through the intact skin and forms a subcutaneous depot. Absorption is mediated by diffusion and is influenced by the thickness of the lipophilic keratinous *stratus corneum* (4, 5). When fentanyl passes through the skin, fentanyl is absorbed into the microcirculation followed by the systemic circulation (1, 4).

A steady state is usually reached after application of a second transdermal fentanyl patch (6), although plasma concentration vary over the 72 hour period wherein a single patch is used (7). Unfortunately, there is a wide intra- and interpatient pharmacokinetic variation in patients using fentanyl patches (7-11). In clinical practice patients may already describe less painkilling effects of the cutaneous patch after 48 hours, and they may use extra opioids in the last 24 hours. Or they need to change their cutaneous patch every 48 hours leading to extra costs which are not always reimbursed by the insurers company. Despite the fact that numerous factors have been investigated, this variation is still largely unexplained (8). The area under the curve (AUC) of fentanyl increased up to 3-fold in volunteers who used strong CYP3A4 inhibitors (like troleandomycine or ritanovir) together with fentanyl (12-16). The combination of the moderate CYP3A4 inhibitor fluconazole and fentanyl showed a significant decrease in clearance of fentanyl (15).

Patients with cancer commonly require polypharmacy to treat side effects of (chemo-) therapy, complications of the underlying cancer or other diseases. Pharmacokinetic drug-drug interactions in cancer patients are therefore highly relevant (17, 18). This is further emphasized by two case reports describing severe and even lethal fentanyl intoxications after a drug-drug interaction between fentanyl and fluconazole or itraconazole, respectively (19, 20). Further study on the concurrent use of CYP3A4 inhibitors and fentanyl is therefore warranted. Aprepitant is deemed a moderate CYP3A4 inhibitor. It is the first neurokinin-1 (NK-1) receptor antagonist and it is used in combination with a 5-hydroxytryptamine-3 ($5HT_3$) antagonist and dexamethasone for the prevention of nausea and vomiting in case of highly or moderately emetogenic chemotherapy (21, 22). Both aprepitant and fentanyl are thus widely and simultaneously used in cancer patients and because of aprepitant's inhibitory capacity on CYP3A4, it could hypothetically increase the exposure of fentanyl, leading to more side effects like nausea or sleepiness. Nonetheless, higher systemic fentanyl concentrations could also lead to a better control of pain. Nevertheless, clinicians should always be aware of potential drug-drug interactions with fentanyl and more frequently monitor pain and side effects in these patients unexplained (8).

Another factor that may influence fentanyl exposure is the localization of the patch on the skin. Now, a fentanyl patch is advised to be applied on dry, intact, skin of the trunk, upper arm, or leg. Most patients prefer the upper arm. When changing the patch, it always has to be applied at another place because of the subcutaneous depot. However, also the localization where the fentanyl patch is applied may influence fentanyl absorption due to differences in skin thickness and/or the amount of subcutaneous fat. Two previous studies measured the residue in used patches of patients with cancer. Comparison of 100 patients showed a 7.5% lower delivery efficiency of fentanyl for patches applied to the leg in comparison to the arm (23). The other study showed no differences in fentanyl absorption between patches applied to arm, shoulder, chest and back (11). However, in both studies plasma fentanyl concentrations were not measured and both studies used inter patient comparisons, making the conclusions less robust given the high interpatient variation mentioned above.

We hypothesized that because fentanyl is highly lipophilic, higher plasma concentrations will be reached when the patch is used on areas with thicker skin, as they usually contain more fat. Mean skin thickness of the upper arm and the upper back are almost equal (43.9 µm versus 43.4 µm),while the mean skin thickness of the thorax is less (37.6 µm) (24). Therefore, we expected differences in fentanyl concentrations between the upper arm/ upper back and the ventral thorax region for sticking the fentanyl patch. For convenience of the patient we choose to compare the upper arm with thorax region for the transdermal delivery of fentanyl in the current study.

In this report we describe the results of these two studies in which the effect of the concomitant use of aprepitant and the localization of the patch on the exposure to fentanyl were investigated.

2 Methods

The two studies were performed as single-center pharmacokinetic studies at the Erasmus MC Cancer Institute. Inclusion criteria were similar for the two studies: patients with cancer, age ≥ 18 years, using a stable dose of a transdermal fentanyl (Durogesic®) for at least 8 days irrespective of the dose used and given written informed consent. Exclusion criteria were: use of fentanyl rescue medication (other opioids were allowed), the use of strong CYP inhibitors or inducers (25) and evidence of serious psychiatric illness, confusion or intellectual disability.

Aprepitant study

This study used a randomized cross-over design with two study periods, each lasting eight days. In both periods patients used a stable dose of fentanyl, whereas patients were randomized for the use of aprepitant between arm A and arm B (**Figure 1)**.

Patients in arm A used aprepitant in the first study period, whereas patients in arm B used aprepitant in the second study period. Patients applied the patch alternately to the right and left upper arm, with a new patch on day one of each study period. The patch was changed every 3 days (72 hours), according to label instructions. Aprepitant was used in the order: 125mg-80mg-80mg on day 6, 7 and 8 of the study period, respectively, concurrently with the fentanyl patch. Pharmacokinetic sampling

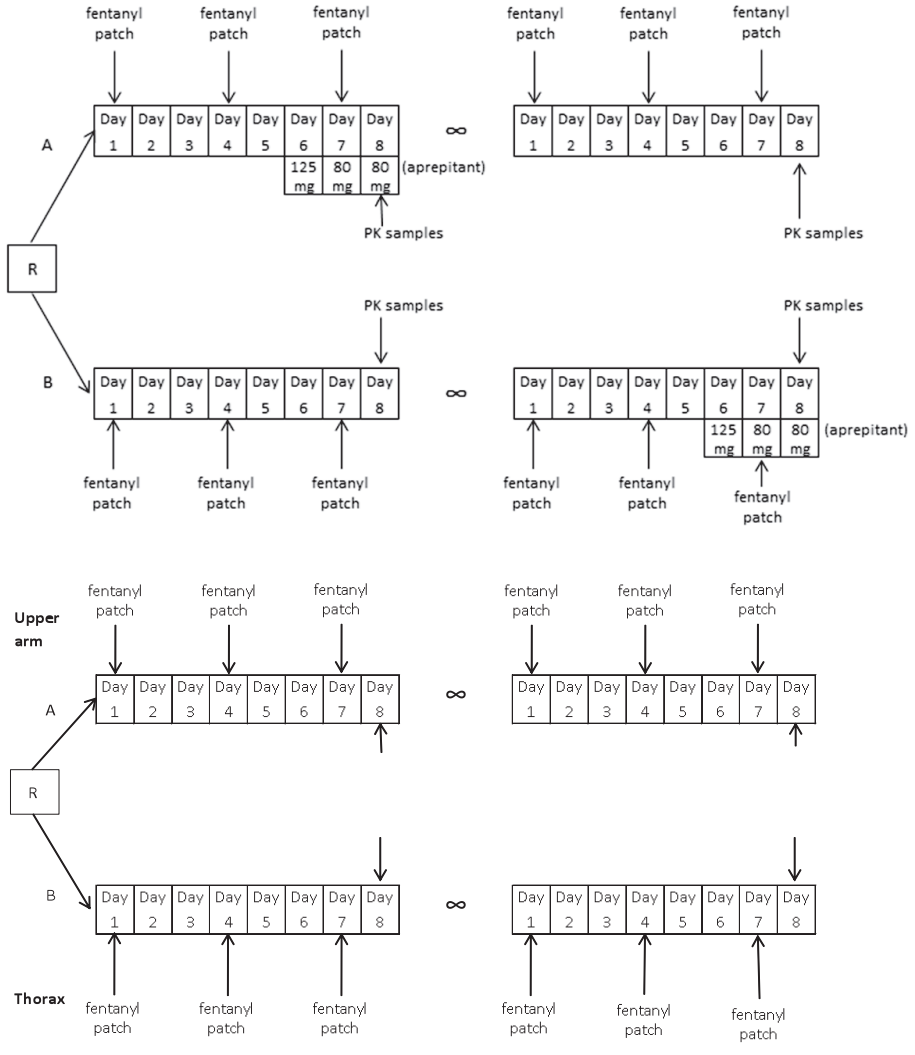

Figure 1 Study design of aprepitant study and patch localization study.

was performed at day 8, approximately 24 hours after changing a patch. Venous blood samples were taken at baseline (just before taking aprepitant) and at 2, 3, 4, 5 and 6 hours after administration of aprepitant or at similar times for the periods in which aprepitant was not used. Blood samples were collected in potassium EDTA coated tubes.

The following routine chemistry data were collected: aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, albumin and alkaline phosphatase (ALP), creatinine, estimated glomerular filtration rate (eGRF), calculated by Modification of Diet in Renal Disease (MDRD); formula: eGFR (mL/min/1.73 m^2) = 32788 x serum Creatinine (µmol/L) -1.154 x age (years) -0.203 x (0.742 when female) x (1.210 when of African descent).

Patch localization study

This study used a randomised cross-over design with two eight-day study periods as well **(Figure 1)**. According to randomisation patients applied the patch to the upper arm (group A) or thorax (group B) first. The patch was changed every 3 days to the opposite arm or thorax, according to regular use. Pharmacokinetic sampling was performed at day 8, approximately 24 hours after changing a patch. Three venous blood samples were collected, with 2 hours between each sample. After collection of the blood samples patients switched to the other patch localization, either thorax or upper arm dependent on randomization. The same sampling procedure as during the first study period was followed.

2.1 Measurements of fentanyl plasma concentrations

We quantified fentanyl in EDTA plasma. A validated UPLC-MS/MS method. This method consisted of a Waters Acquity UPLC sample manager, coupled to a triple quadruple mass spectrometer operating in the multiple reaction monitoring mode (MRM) with positive ion electro spray ionization (Waters, Etten-Leur, The Netherlands). The multiple reaction monitoring transitions was set at 337⇃188.

Chromatographic separations for fentanyl were achieved on an Acquity UPLC® BEH C18 1.7 µm 2.1 x 100 mm column eluted at a flow-rate of 0.350 mL/min on a gradient of methanol. A cycle time for this method was about 6 minutes. Calibration curves were linear over a wide range (0.100 to 10.0 ng/mL) with at lower limit of quantitation (LLQ) of 0.100 ng/mL for fentanyl. The within and between-run precisions, including the LLQ, were ≤ 5.52 % and ≤ 6.12 %, respectively, while the average accuracy ranged from 86.2 % to 97.5%. The extraction of 200 µL of plasma involved a deproteinization step with acetone, followed by a simple liquid extraction with ethyl acetate. The organic phase was evaporated and subsequently dissolved in 100 µL methanolic solutions, from which aliquots of 10 µL were injected into the UPLC-MS/MS system.

2.2 Statistics

For both studies (1. the combination of aprepitant and 2. the localization of the fentanyl patch) a difference in systemic exposure to fentanyl of 30% was determined to be clinically relevant. It was assumed that the intra-patient relative standard deviation in fentanyl pharmacokinetics was 20%. Given a power of 80%, 14 patients were required in each study to detect a difference. For the primary endpoint, the following analysis approach was taken. A natural log transformation was applied to the $AUC_{0\text{-}4h}$ and $AUC_{0\text{-}6h}$ values in order to normalize the distributions (4, 7, 26). Estimates for the mean differences in (log) AUCs were obtained using a linear mixed effect model with treatment, sequence and period as fixed effects and subject within sequence as a random effect (27). Variance components were estimated based on restricted maximum likelihood (REML) methods and the Kenward-Roger method of computing the denominator degrees of freedom was used. The mean differences and 95% CIs for the differences were exponentiated to provide point estimates of the ratio of geometric means and 95% CIs for these ratios, which can be interpreted as relative differences in percentages. Regular chemistry results, which were measured only in the aprepitant study, were compared between periods by means of the Wilcoxon signed rank test.

2.3 Compliance with ethical standards:

The two studies were approved by the medical ethics review board (aprepitant study; MEC 13.387 and patch localization study; MEC 12.193) and conducted in accordance with the Declaration of Helsinki. The trials were registered in the Dutch Trial Register (Trial registration ID: aprepitant study: NTR4288; localization study NTR3654). Written informed consent was obtained from all patients.

3 Results

Aprepitant study

A total of 20 patients was included, while 6 patients were withdrawn from the study before start of PK sampling because of a deteriorated condition. As a result, 14 patients (6 females and 8 males) with a median age of 61 years (IQR 55-71) completed the study and were evaluable. Unfortunately, two patients had missing PK measurements at the 6 hour time point. The demographic data of the evaluable patients are presented in **Table 1**.

No significant differences were found in the chemistry results between period 1 and 2, and therefore did not affect the outcomes of the study.

The $AUC_{0\text{-}6h}$ was 7.1% (95% CI: -28% ; +19%) lower when fentanyl was used in combination with aprepitant as compared to using fentanyl without aprepitant. The

inter- and intra-patient coefficients of variation in fentanyl were 59% and 28%, respectively. Log-transformed fentanyl concentrations are shown in **Figure 2**. AUC_{0-4h} analysis was also performed and showed the same non-significant results (relative difference in AUC_{0-4h} was 4.5% (95% CI: -24%; +20%, fentanyl with aprepitant in comparison to fentanyl without aprepitant).

Table 1 Patient characteristics in aprepitant study

		N=14
Sex, n - Male - Female		 8 6
Age in years (median and IQR)		60.5 (55-71)
Height in cm (median and IQR)		172.5 (167-180)
Weight in kg (median and IQR)		71 (67-92)
BMI (median and IQR)		26 (19.5-29.5)
Fentanyl patch dose (µg/h) mean (range)		25 (12-43.5)
Laboratory results (n=12) (median (IQR) (normal range)	Period 1	Period 2
Creatinine (55-90 µL/min)	67 (62-92)	67 (61-86)
MDRD (> 60 mL/min/1,73 m2)	87 (66-90)	85 (70-90)
AST (< 31 U/L) (n=11)	30 (20-47)	26 (21-45)
ALT (< 34 U/L)	23 (14-41)	24 (12-40)
Bilirubin (<17 umol/L)	7 (4-12)	8 (4-12)
ALP (< 98 U/L) (n=11)	133 (87-282)	133 (90-247)

Patch localization study

Twenty-three patients were included. Fourteen patients (11 females and 3 males) with a median age of 62 years (IQR 57-65) completed the study and were evaluable. The demographic information about these patients is presented in **Table 2**. The other nine patients were not evaluable due to clinical deterioration and missed blood sampling for pharmacokinetic analyses. The AUC was 7.4% (95% CI: -22% - +49%) higher when the patch was applied to the upper arm as compared to the thorax. The inter- and intra-patient coefficient of variation in fentanyl (normalized AUC) were 48% and 41%, respectively. **Figure 3.**

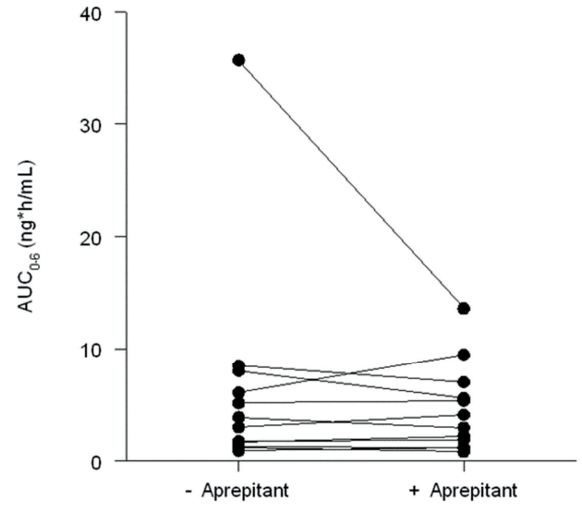

Figure 2 Plasma fentanyl concentrations (AUC_{0-6h}) aprepitant vs no aprepitant.

Table 2 Patient characteristics in patch localization study

	N=14
Sex, n - Male - Female	 3 11
Age, years (median and IQR)	62 (57-65)
Height, cm (median and IQR)	167 (162-172)
Weight, kg (median and IQR)	66 (63-78)
BMI (median and IQR)	23.6 (22.6-28.0)
Fentanyl patch dose (µg/h) mean (range)	52.5 (12-175)

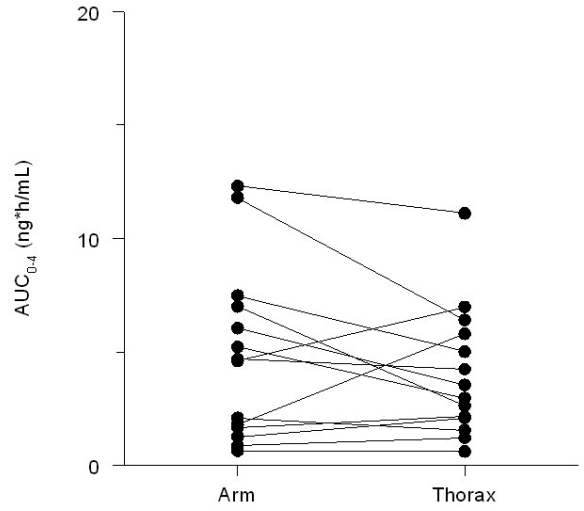

Figure 3 Plasma fentanyl concentrations (AUC_{0-4h}) upper arm vs thorax.

4 Discussion

The concomitant use of aprepitant for 3 days showed no statistically significant influence on the AUC of fentanyl in patients with cancer using transdermal fentanyl. Neither did the localization of the fentanyl patch.

Several studies investigated drug-drug interactions with aprepitant before, because of its ability to inhibit CYP3A4 (28). However only one study investigated drug-drug interactions with aprepitant and opioids (29). Concomitant use of aprepitant and oxycodone in patients with cancer led to a 25% higher AUC of oxycodone (29). Therefore, it is surprising that a drug like aprepitant, that is highly metabolized by CYP3A4 did not increase the exposure of fentanyl in a clinically and statistically significant extent. To limit the number of sampling moments in our patients, we have limited our study to just 1 course aprepitant of 3 days. The effect of multiple courses would have been interesting.

Studies, as mentioned in the introduction, with strong CYP3A4 inhibitors did result in increased fentanyl exposure, although the effect sizes in those reports were smaller than theoratically expected (8, 12, 14, 16). A previous study with fluconazole, a moderate CYP3A4 inhibitor like aprepitant, showed a significantly lower clearance of fentanyl (11.6 +/- 3 mL/min/kg vs 14.0 +/- 2.5 mL/min/kg) when used together, but no significant difference on AUC. In our study only AUC was measured, so possible effects on other pharmacokinetic parameters are unknown.

The most accepted hypothesis of fentanyl metabolism is that fentanyl is mainly metabolized in the liver by CYP3A4 mediated N-dealkylation resulting in the inactive metabolite norfentanyl (4, 30, 31). However, a recent study showed that other unknown metabolic routes might also play a role in fentanyl metabolism and that the N-dealkylation step might be less predominant than previously thought (16), thereby possibly explaining the limited influence of aprepitant on fentanyl exposure. In that study, the metabolic clearance of fentanyl to norfentanyl was strongly inhibited by ketoconazole, but only a small increase of fentanyl exposure in general was seen (16). For future research it would be interesting to study the different metabolites in plasma and urine to see whether aprepitant has an impact on the formation of those metabolites.

We studied only the combination of aprepitant with transdermally applied fentanyl. Several rapid onset forms of fentanyl are now available, and we cannot exclude that there will be an effect of aprepitant on these formulations. Previous studies with CYP3A4 inhibitors and fentanyl used mostly intravenously administrated fentanyl (14-16, 32, 33). Of the rapid onset opioids only transmucosal fentanyl citrate has been studied (31). The combination of the strong CYP3A4 inhibitor troleandomycine showed in both intravenously and transmucosally administrated fentanyl higher AUC's compared to fentanyl alone (31, 32). Since effect sizes of CYP3A inhibitors may be different among various administration routes, the results of the current analysis cannot be extrapolated to rapid onset opioids. Therefore, extra attention is needed when aprepitant is prescribed to patients who also use fentanyl rapid onset opioids . In this study the localization of the fentanyl patch did not statistically significantly influence the exposure to fentanyl. An interpatient comparison in another study investigating fentanyl delivery, by analyzing patches, between patients applying patches to the leg versus the thorax found a small non-significant 7.5% difference in favor of the arm (23). Our intra-patient comparison showed a similar (non-significant) difference between the arm and the upper thorax. Unfortunately, actual skin thickness or other characteristics describing skin condition were not measured in our study. Despite that, our study describes the situation in daily clinical care and is therefore of relevance for both patients and physicians. This study demonstrated that skin thickness is of minor importance for transdermally delivered fentanyl.

The inter individual variation in plasma fentanyl levels were much larger than we had expected. Therefore our studies were underpowered to find a clinically and statistically significant difference of 30% in the AUC's.

5 Conclusion

In these two cross-over studies we could not identify any effect of aprepitant or the localization of the patch on fentanyl pharmacokinetics.

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6

Pharmacokinetics of Sublingually Delivered Fentanyl in Head and Neck Cancer Patients Treated with Curatively Aimed Chemo or Bioradiotherapy

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Abstract

Over 90% of patients treated for head and neck cancer with curatively aimed chemo or bioradiotherapy will develop painful mucositis and xerostomia. Sublingually delivered fentanyl (SDL) is a rapid acting opioid to treat breakthrough pain. It is unclear how SDL is absorbed by the mucosa of these patients. Therefore, the aim of this study was to investigate the effects of mucositis and xerostomia on the absorption of SDL. Thirteen patients who received chemo or bioradiotherapy (RT), were given a single dose of fentanyl: Before start of RT, 3 and 6 weeks after start of RT, and 6 weeks after finishing RT. Pharmacokinetic samples were taken. The primary endpoint was the relative difference (RD) between systemic exposure to fentanyl (area under the curve; AUC) at baseline ($AUC_{baseline}$) and fentanyl AUC in the presence of mucositis grade ≥2. The secondary endpoint was the RD between $AUC_{baseline}$ and fentanyl AUC in the presence of xerostomia, which were analyzed by means of a paired t-test on log-transformed data. Mucositis resulted in a 12.7% higher AUC (n = 13; 95% CI: −10.7% to +42.2%, p = 0.29) compared to baseline levels and xerostomia resulted in a 22.4% lower AUC (n = 8; 95% CI: −51.9% to +25.3%, p = 0.25) compared to baseline levels. Mucositis grade ≥2 or xerostomia caused by chemo or bioradiotherapy does not significantly alter the systemic exposure to SDL. Patients with pain during and after chemo or bioradiotherapy may be safely treated with SDL.

1 Introduction

For patients with head and neck cancer, combined treatment strategies consisting of radiotherapy and cisplatin (chemoradiotherapy) or cetuximab (bioradiotherapy), respectively, have been reported with improved local tumor control and survival rate in comparison to radiotherapy alone (1-3). The consequence of this combined treatment, however, is a higher incidence of severe and dose-limiting side effects during and after therapy. This is especially the case for mucositis and xerostomia (1-4).

Mucositis is an inflammatory process of the mucosa characterized by erythema, inflammation, and/or ulceration of the mucosa due to tissue damage (4, 5). Reported overall incidence rates of mucositis after chemoradiotherapy or bioradiotherapy are 97% and 93%, and for common terminology criteria (CTC) grade ≥3 mucositis 34% and 56%, respectively (6). Mucositis may be associated with severe pain, weight loss, need for a feeding tube, hospitalization, and, as a result, increased medical costs (7). Especially, cumulative doses of >39 Gy were associated with severe mucositis (8).

During combined therapy, the severity of mucositis gradually increases, and most patients require analgesics—mostly opioids—from the third week of treatment until 2 to 6 weeks after the last radiotherapy dose (4, 9). Due to mucositis and associated swallowing problems, the use of oral medication can be difficult and painful (10, 11). Therefore, transdermal opioids are preferred in these patients for the treatment of severe chronic pain (10, 12) and transmucosal products may be good candidates for the management of breakthrough pain (13, 14).

The sublingual fentanyl tablet (Abstral®) is one of the transmucosal rapid onset opioids (ROOs). ROOs are well tolerated and may provide more efficacious treatment than oral morphine in patients suffering from breakthrough pain (14-16). In clinical studies, patients experienced a significant pain relief after administration of sublingual fentanyl within 15 min (17). Pharmacokinetic studies showed a fast increase in plasma concentrations after the administration of sublingual fentanyl with the first quantifiable drug concentrations (T_{first}) found between 8 and 15 min, whereas the time to peak concentration (T_{max}) varied from 30 min to 2 h (18, 19). Although a wide variation in pharmacokinetics is known for all fentanyl products, this variation is still largely unexplained (20). Factors that might potentially influence absorption are of extra importance in transmucosal administration.

Currently, there are no data available on the use of sublingually delivered fentanyl in clinically relevant mucositis (grade 2 or higher) (6). Therefore, it is unknown if mucositis influences the bioavailability of sublingually delivered fentanyl. A previous pilot study showed a trend towards a higher exposure to buccally delivered fentanyl in patients with mucositis compared to patients without mucositis (21), while another study showed no differences (22). However, because of wide inter-individual variations in the pharmacokinetics of (transmucosal) fentanyl, cross-sectional studies

may not be most appropriate to study effects of mucositis on fentanyl exposure. Therefore, we set up a prospective study in patients with head and neck cancer treated with chemo or bioradiotherapy.

As mentioned, xerostomia is another important side effect of chemo or bioradiotherapy and is mainly due to irradiation of the salivary glands. The severity of xerostomia is maximal at 6 weeks after the start of radiotherapy, but remains severe until 6 months after the last dose (23). Because of the potential influence of xerostomia on the uptake of sublingually delivered fentanyl, we also investigated the systemic exposure to fentanyl six weeks after the end of the chemo or bioradiotherapy. At that time the intensity of xerostomia is still severe, but mucositis has resolved substantially in most patients (23, 24).

2 Methods

2.1 Patients

A single-center pharmacokinetic study was carried out at the Department of Medical Oncology of the Erasmus MC Cancer Institute between October 2014 and January 2017. The study was approved by the local medical ethics review board at November 26th 2013 and conducted in accordance with the latest version of the Declaration of Helsinki. The trial was registered at the Dutch Trial Registry (www.trialregister.nl ID: NTR4995). Patients of ≥18 years with head and neck cancer planned for curatively aimed radiotherapy with cisplatin or cetuximab were considered for inclusion in the study. Exclusion criteria included the use of fentanyl medication within one week before inclusion in the study (other opioids and non-opioid analgesics were allowed), opioid intolerance, former allergic reactions to opioids, serious psychiatric illness, confusion, intellectual disability, or earlier lymph nodes dissection in the head/neck region. The use of cytochrome P450 (CYP) inhibitors was allowed when there was no indication to change the dose of that drug during the study. Dexamethasone and aprepitant were allowed as standard anti-emetic therapy for patients treated with cisplatin. All enrolled patients provided written informed consent.

2.2 Study Design

Patients were given a single dose of 200 µg fentanyl (Abstral®) sublingually at 4 different time points in their regular treatment schedule of chemo or bioradiotherapy. Before administration of the fentanyl, patients had to rinse their mouth. The first dose of fentanyl was given before the start of the radiotherapy (baseline), the second dose 3 weeks after starting radiotherapy (T_1), the third dose 6 weeks after starting radiotherapy (T_2) and the last dose 6 weeks after finishing radiotherapy (T_{last}). In case of chemo-radiotherapy, the fentanyl dose was planned 24 to 72 h before cisplatin treatment to avoid interference with the used CYP3A4 inhibiting or inducing antiemetic medication, e.g., aprepitant and dexamethasone.

Radiotherapy consisted of 70 Gy in 35 fractions of 2 Gy to the primary tumor and clinically relevant positive nodes during a period of 6 to 7 weeks. Cisplatin (100 milligram per square meter (mg/m^2) was given at day 1, 22, and 43 of the radiotherapy. Cetuximab (250 mg/m^2) was given weekly during radiotherapy preceded by a loading dose (400 mg/m^2) a week before start of the radiotherapy.

When patients needed analgesics, they could use all opioids except fentanyl products. When fentanyl was deemed necessary, patients left the study and were replaced.

2.3 Blood Sampling and Measurement of Fentanyl Concentrations

Pharmacokinetic (PK) samples were taken pre-dosing, and at 10, 20, 30, 40, 50, 60, 90, 180, and 360 min after administration of sublingual fentanyl.

Blood samples (4.5 mL) were collected in potassium ethylenediaminetetraacetic acid (EDTA) coated tubes and centrifuged for 10 min at 2500 to 3000× g at 4 °C. Plasma was transferred into polypropylene tubes (1.8 mL Nunc vials), which was stored at T < −70 °C (T < −20 °C during collection period) until the time of analysis. Fentanyl in plasma was quantitated using a validated UPLC-MS/MS method (25).

Pharmacokinetic data were analyzed by using Phoenix WinNonlin version 7.0 (Certara, Princeton, NJ, USA) to analyze concentration-versus-time data. Peak concentration (C_{max}), time to peak concentration (T_{max}), and area under the concentration-time curve (AUC) from 0 to 6 h after administration, were calculated.

2.4 Clinical Assessments

Mucositis, xerostomia, pain, and general toxicity were measured prior to the administration of sublingual fentanyl. Mucositis was scored with CTCAE 4.03 toxicity criteria (6), xerostomia with the Groningen Radiotherapy-induced Xerostomia questionnaire (GRIX) (26) and pain with the Numerical Rating Scale (NRS) (27). When patients suffered from moderate-severe pain (NRS ≥ 4) at the start of the PK sampling, then pain was also assessed at the PK sampling time points.

Other toxicities, i.e., nausea, vomiting, anorexia, dizziness, drowsiness, and fatigue, were also scored with CTCAE 4.03 prior to, and one hour after, the administration of sublingual fentanyl.

2.5 Statistical Considerations

2.5.1 Sample Size Calculation

The primary outcome measure was fentanyl AUC. A relative difference of 25% between the AUC at day 1 ($AUC_{baseline}$) and the AUC at the first moment a mucositis with a severity of at least CTC grade 2 (AUC_{muco}) was found during chemo of bio-

radiotherapy was considered as clinically relevant. Assuming a within-patient variability in AUC of 20%, 13 evaluable patients were needed to obtain 80% power (2-sided significance level ↓ = 0.05) to detect a difference (28).

2.5.2 Statistical Analyses

The difference in AUC between day 1 and the first moment with a mucositis grade ≥2 (for each individual patient determined) was analyzed by means of a paired t-test. Since it was assumed that the AUC follows a log-normal distribution, analyses were performed on log-transformed data. The results were then back-transformed by taking the exponentials from the difference and corresponding 95% confidence interval, which represents the ratio of the geometric means and can be interpreted as the percentage of change (i.e., relative difference (RD)) between AUC_{muco} and $AUC_{baseline}$. A similar approach was used for the analysis of C_{max}. Differences in T_{max} were analyzed using a Wilcoxon signed rank test.

For the analysis of the effect of xerostomia on the PK of sublingual fentanyl, differences in AUC, C_{max}, and T_{max} were analyzed in the same way as the analysis for mucositis.

3 Results

Fourteen patients were included of whom 13 patients (11 males and 2 females) were evaluable. One male patient was excluded for further analysis due to protocol violation by accidentally receiving fentanyl analgesic therapy outside the study protocol. The demographic data of these evaluable patients are presented in Table 1. The median age was 62 years (range 48 to 72). Patients were treated for cancer of the oropharynx (n = 4), hypopharynx (n = 4), larynx (n = 4), or combined oropharynx/larynx cancer (n = 1). Nine patients presented with cervical lymph node metastases. In all patients CTC grade ≥2 mucositis was diagnosed during the treatment with chemo or bioradiotherapy; in nine patients at 3 weeks, and in the other four patients at 6 weeks after the start of the treatment. The cumulative radiotherapy doses at T_1 and T_2 are given in Table 2.

3.1 Analyses for Mucositis

The geometric mean $AUC_{baseline}$ was 1.04 ng/mL*h (coefficient of variation (CV) = 41.7%) and the $AUC_{mucositis}$ was slightly higher: 1.18 ng/mL*h (CV = 36.1%). This was a relative difference of 12.7% (95% CI: −10.7% to +42.2%, p = 0.29; see Figure 1). The geometric mean of the maximum concentration (C_{max}) of fentanyl at baseline was 0.43 ng/mL*h (CV = 40.0%), and C_{max} mucositis was 0.45 ng/mL*h (CV = 64.3%). This is a relative difference of 5.1% (95% CI: −28.1% to +53.8%, p = 0.78) (Figure 2A). The median time to reach C_{max} (T_{max}) after administration of fentanyl was

Table 1 Patient characteristics.

Variable	Total N = 13
Sex, n (%)	
Male	11
Female	2
Age, years (median and range)	62 (48–72)
BMI (median and IQR)	25.4 (22.8–26.9)
Tumor type	
- oropharyngeal carcinoma	4
- hypopharyngeal carcinoma	4
- laryngeal carcinoma	4
- combined oropharyngeal and laryngeal carcinoma	1
Concurrent to radiotherapy	
Cisplatin	5
Cetuximab	8
Laboratory results (median (IQR) (normal range)	
- Creatinine (55–90 µL/min)	79.0 (78.0–90.0)
- MDRD (>60 mL/min/1.73 m²)	82.5 (73.0–87.5)
- AST (<31 U/L)	24.5 (22.0–34.0)
- ALT (<34 U/L)	40.5 (21.0–48.0)
- Bilirubin (<17 µmol/L)	6.0 (5.0–7.0)
- Albumin (35–50 g/L)	41.5 (41.0–46.0)
- ALP (<98 U/L)	80.5 (62.0–100.0)

Abbreviations: BMI; body mass index, IQR; interquartile range, MDRD; modification of diet in renal disease, AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase.

Table 2 Radiotherapy dose during the chemo- or bioradiotherapy.

Radiotherapy dose and fentanyl AUC	$T_{baseline}$	T_1	T_2	$T_{mucositis\ T1}$ n = 9	$T_{mucositis\ T2}$ n = 4
Radiotherapy dose sublingual in Gy (mean, SD)	-	13.2 (6.7)	28.2 (12.9)	12.9 (4.4)	40.1 (SD 13.7)
Radiotherapy dose total in Gy (mean SD)	-	30.8 (6.2)	55.6 (4.7)	32.7 (6.7)	54.3 (1.3)
Fentanyl AUC ng/mL*h geometric mean (CV %)	1.04 (41.7)	1.09 (40.6)	1.31 (42.2)	x	x

Legend: T_1 = 3 w after start of radiotherapy; T_2 = 6 w after start of radiotherapy.

40 min (range 10 min to 1 h and 35 min) at baseline, and T_{max} mucositis was 30 min (range 10 min to 1 h and 3 min), which did not differ significantly (p = 0.62).

3.2 Analyses for Xerostomia

Measurements at 6 weeks after finishing radiotherapy were available for eight patients. The other patients withdrew consent after finishing radiotherapy. In six out of eight evaluable patients, the GRIX score had increased for all four domains (Table 3). The geometric mean AUC of fentanyl at baseline in these eight patients was 1.12 ng/mL*h (CV = 45.1%) and at T_{last} this was 0.87 ng/mL*h (CV = 49.3%). This is a relative difference of −22.4% (95% CI: −51.9% to +25.3%, p = 0.25). The geometric mean of C_{max} of fentanyl at baseline was 0.44 ng/mL*h (CV = 44.4%) and at T_{last} this was 0.31 ng/mL*h (CV = 66.2%). This is a relative difference of −29.7% (95% CI: −64.6% to +39.7%, p = 0.27; see Figure 2B). T_{max} after administration of fentanyl was 40 min (range 20 min to 1 h and 35 min) at baseline, and 60 min (range 20 min to 1 h and 31 min) at T_{last}, which did not differ significantly (p = 0.36).

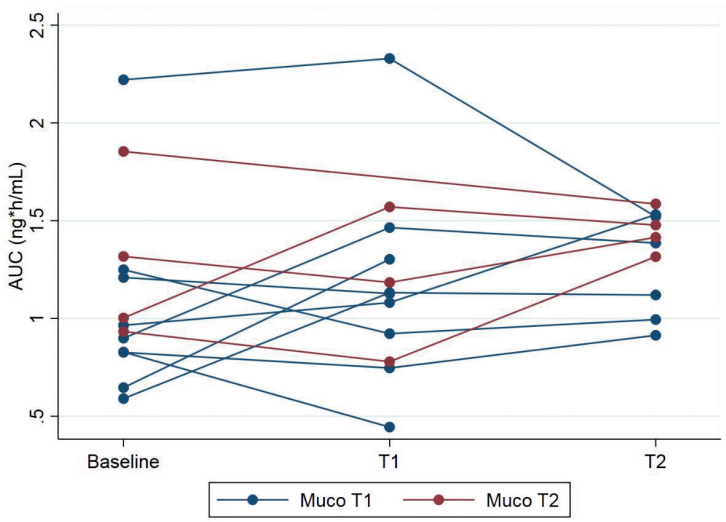

Figure 1 Individual area under the curve (AUCs) plotted against three time points; baseline, 3 weeks (T1) and 6 weeks (T2) after start. Each line represents an individual patient. Patients with a blue line had grade ≥2 mucositis at T1 and patients with a red line at T2.

Table 3 Xerostomia analysis measured by GRIX.

GRIX score	Baseline (n = 8) 0–100	T_{last} (n = 8) 0–100
Day xerostomia Median (IQR)	11.11 (0.00–22.22)	38.89 (22.22–77.78)
Day sticky saliva Median (IQR)	0.00 (0.00–11.11)	27.78 (0.00–61.11)
Night xerostomia Median (IQR)	22.22 (5.56–27.78)	38.89 (33.33–66.67)
Night sticky saliva Median (IQR)	0.00 (0.00–8.33)	16.67 (0.00–66.67)

Legend: IQR: interquartile range.

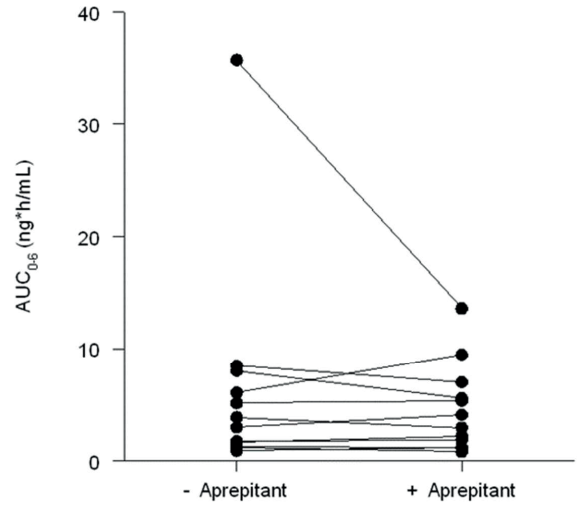

Figure 2 (**A**) The mean concentration time curve of fentanyl in patients with (○) and without (●) mucositis grade ≥ 2. (**B**) The mean concentration time curve of fentanyl in patients with (○) and without (●) xerostomia.

3.3 Analysis of Pain

Pain was measured before every administration of the fentanyl. Only if pain scores were ≥4, pain scores were continued during sampling time. At 15 of the 52 pain measurements before administration of fentanyl pain intensity was ≥4. These 15 high pain scores occurred in 10 out of 13 patients. Three patients experienced pain at

baseline, nine patients after onset of mucositis and three patients at T_{last}. The median decrease in pain intensity after the administration of 200 µg fentanyl was 2 (range 0 to 8).

3.4 General Toxicity

Only two patients experienced dizziness and drowsiness CTC grade 1 after the administration of sublingual fentanyl. No other toxicities were seen due to administration of sublingual fentanyl.

4 Discussion

This is the first study that investigated the effects of mucositis and xerostomia on the pharmacokinetics of sublingually administered fentanyl in patients treated with chemo or bioradiotherapy for head and neck cancer. We found no significant differences in the exposure to sublingually delivered fentanyl in patients with a clinically relevant mucositis grade 2 or higher compared to their own baseline values. This is in line with two studies in patients with cancer that investigated the influence of mucositis (CTCAE grade 1) on buccally delivered fentanyl in an inter-patient comparison (21, 22). The major strengths of our study are the intra-patient comparisons and the standardized measurements in time.

Patients suffered from general erythema and oedema in the mouth, but not specifically under the tongue. Not all patients reached a dose of 39 Gy sublingually; the dose which has been correlated with severe mucositis (Table 3) (8). The most severe mucositis is likely to occur in the radiotherapy area around the tumor (oropharynx, hypopharynx, and larynx) and the pathologic cervical lymph nodes. Therefore, the results of this study cannot be (simply) extrapolated to patients with moderate to severe mucositis caused by chemotherapy alone, since chemotherapy induced mucositis is typically located in the mouth and not only in the pharyngeal and laryngeal parts (29-31). In addition, the within-patient variability in AUC was higher than expected beforehand, and therefore this outcome resulted in a lower power to detect a (potential) difference. This is a weakness of our study, and therefore the study may be assumed as a pilot study.

The increase in xerostomia we found after chemo or bioradiotherapy is in line with other studies (3, 26, 32). Yet, our study was not powered to find significant differences in fentanyl pharmacokinetics between baseline and post-treatment measurements with xerostomia. We found 22% and 30% lower geometric means of respectively AUC and C_{max} during xerostomia compared to baseline. However, these differences were not statistically significant. Contrasting results were found in a study in patients with salivary gland hypofunction (33). Moistening the mouth with water or pilocarpine hydrochloride (a cholinergic agonist), before taking sublingually delivered fentanyl, led to higher C_{max} and shorter T_{max} compared to the situation of xerostomia without moistening (33). Our study results might be explained by moistening the mouth before every sublingual fentanyl administration.

Most patients were adequately treated with pain medication. Therefore, the number of measured episodes in which pain was assessed as ≥4 was low. The median decrease in pain intensity was 2, which is similar to studies on the use of sublingual fentanyl for breakthrough pain in patients with cancer (34, 35). This clinical effect is in line with the stable pharmacology we found during the chemo or bioradiotherapy. Although our patients suffered from mucositis, the results of sublingual fentanyl on pain seems to be comparable to all these studies.

In this study, sublingually delivered fentanyl was administrated to opioid naïve patients while it is registered for non-opioid naïve patients. Additionally, to ensure quantification of fentanyl plasma levels up to 8 h post dose, a higher dose of 200 mcg was given instead of the standard starting dose of 100 mcg. The higher starting dose did not led to any serious side effects in any of our opioid naïve patients.

Based on the findings in this study, we may provide some practical recommendations to physicians and patients. Sublingual fentanyl is a convenient option to treat breakthrough pain in patients with mucositis caused by chemo or bioradiotherapy. The uptake of sublingual fentanyl in patients with local ulcers or xerostomia is unknown and might be affected, and thus requires close monitoring of the effect. Moisturizing the mouth in case of xerostomia is recommended before the administration of fentanyl.

5 Conclusions

Mucositis grade 2 or higher, caused by radiotherapy in combination with cisplatin or cetuximab, did not significantly influence the systemic exposure to sublingually delivered fentanyl. Xerostomia led to non-significant lower AUC values (30%) and fentanyl concentrations (22%) compared to baseline. Therefore, patients with pain during and after chemo or bioradiotherapy for head and neck cancer may be safely treated with sublingually delivered fentanyl.

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7

Summary, discussion, and future perspectives

Summary

Pain is highly prevalent in patients with cancer, especially in patients with advanced malignancies. Pain is treated according to the steps defined in the WHO pain ladder (1). Step 3 in this stepwise approach is the use of strong acting opioids. They are the drugs of choice for moderate-severe pain in patients with cancer. Several strong acting opioids are available, but in this thesis the focus is on fentanyl. The transdermal fentanyl patch is frequently used to treat continuous pain and the oromucosal or intranasal variants are fit to treat breakthrough pain (2-8).

Advantages of fentanyl compared to other opioids are in particular the transdermal administration route to treat continuous pain and a lower incidence of constipation compared to morphine. A systematic review described that constipation was reported as an adverse event by 17% of patients using transdermal fentanyl and by 48% of patients using slow release morphine (9).

Fentanyl is known for its high binding capacity to plasma proteins and its high lipophilicity. (5, 10). Fentanyl is metabolized in the liver and excreted by the kidneys (11, 12). These pharmacological aspects make fentanyl suitable for the mentioned oromucosal and transdermal administration.

An important drawback of fentanyl, however, is its wide inter- and intra-patient variability in pharmacokinetics (7, 13, 14). So, the same dose of fentanyl may not lead to equal plasma levels of fentanyl. Factors that may influence fentanyl pharmacokinetics are currently largely unknown (13, 15-19). Until now fentanyl like other opioids is dosed by titration and dose is slowly increased or decreased until a satisfying effect on pain and acceptable side effects. Nevertheless, during a treatment with a stable dose of fentanyl, episodes of unstable pain and side effects may occur. Some unstable periods may be explained, e.g. in literature fentanyl intoxication has been described in case of the use of a warming blanket, or insufficient analgesia in case of comedication (20-23). However, overall, pharmacokinetic variability is high without a precise understanding which factors contribute to this variability.

The primary aim of this thesis was therefore to better understand the pharmacokinetics of fentanyl in patients with cancer. For this reason we studied the influence of common patient characteristics, prone to change during the cancer disease trajectory in patients with advanced cancer, on fentanyl pharmacokinetics. If we are able to identify factors that influence fentanyl pharmacokinetics in patients, fentanyl doses could be adjusted earlier when interfering factors are recognized. Ideally, this results in less variation in fentanyl plasma levels and possibly a better or safer analgesic response to treatment. The balance between effects and side effects of a

drug is extremely important in a phase of life where it is all about the best quality of life in the sparse time left. In this thesis we started to study the literature to find relevant factors that were studied related to fentanyl pharmacokinetics. Afterwards, we performed prospective studies to study common clinical patients variables prone to change during the cancer disease trajectory in patients with cancer and potentially influence fentanyl pharmacokinetics.

Chapter 2 describes the results of a systematic review of factors studied which potentially influence fentanyl pharmacokinetics. With a systemic Pubmed, Cochrane, and Embase search we identified 31 publications that met the inclusion criteria to describe pharmacokinetic parameters as clearance, Area Under the Curve (AUC) and time to maximum concentration (t_{max}). A total of 36 factors were found and these factors were divided in 4 groups: drug-drug interactions, environmental factors (e.g. local heat added to the patch or localization of the patch), patient related factors (e.g. age, gender, and BMI) and (pharmaco-)genetic variation. We found an enormous heterogeneity in the studies (e.g. research in volunteers instead of patients, small studies versus larger studies, differences in pharmacokinetic sampling). Only a minority of the studies investigated the rapid onset fentanyl products as most studies focused on transdermal fentanyl. None of the studies described pharmacokinetics in relation to clinical effects. After all we identified the use of CYP3A4 inhibitors and inducers, impaired liver function, and heating of the patch as clinically relevant factors in terms of influencing fentanyl pharmacokinetics. The influence of other studied factors was less clear. In elderly patients, data suggested that fentanyl needed to be dosed very carefully due to alterations in absorption and metabolism. The influence of body mass index and gender on fentanyl pharmacokinetics was questionable; most probably due to a large heterogeneity in the published studies. Looking at the influence of pharmacogenetics, e.g. the *CYP3A5*3* gene polymorphism, further studies are warranted to determine its relation with fentanyl pharmacokinetics. Our recommendations for future research were to focus on patients with cancer in various stages of the disease, on patients using various fentanyl products, and on the relationships between pharmacokinetics and clinical effects of fentanyl (pain relief as well as side effects). These recommendations inspired us to perform the prospective studies described in this thesis.

Chapter 3 reports the validation of a laboratory method to measure the opioids morphine, fentanyl, and hydromorphone, and the metabolites norfentanyl, morphine-3ß-glucuronide, and morphine-6ß-glucuronide in human plasma. With a lower limit of quantification for fentanyl of 0.1 ng/mL, this method was suited to support the conduct of the pharmacokinetic studies with fentanyl described in this thesis.

The study described in **chapter 4** is the first study we performed in patients with cancer using transdermal fentanyl to investigate the effects of smoking and body mass index (BMI) on the pharmacokinetics of fentanyl. For this aim we designed an exploratory cohort study with a limited pharmacokinetic sampling strategy. A single blood sample --taken approximately 24 hours after replacing a new fentanyl patch located at the upper arm-- was enough. A total of 88 patients were evaluable, while no statistical differences were found in plasma fentanyl concentrations for the group smokers compared to non-smokers, although data suggested a slightly lower fentanyl exposure in the smoking group. Neither did we find statistical differences for the group of patients with a low BMI (< 20 kg/m^2) compared to the group of patients with a high BMI (> 25 kg/m^2). A caveat of this study was the sample size, as we presumed a lower interpatient variability, based on the proposed fentanyl variability, than we ultimately found in our patient population. This higher PK variation could have influenced the outcome of this study. For the design of future studies in this fragile patient population, one should keep in mind that pharmacokinetic variability may be higher than reported in reference studies with healthier patients or volunteers. This may prevent the conduct of an underpowered study.

In **chapter 5** we simultaneously report the results of two prospective cross-over pharmacokinetic intervention studies. Both studies included a total of 14 patients, both studies were intra-patient comparisons, and both studies were performed in patients with cancer using transdermal fentanyl patches to treat their pain. The first study investigated the influence of the use of aprepitant on the exposure to fentanyl. Aprepitant is a moderate CYP3A4 inhibitor and a common drug to treat and prevent nausea and vomiting caused by chemotherapy. The hypothesis was that aprepitant could have an inhibitory effect on CYP3A4 and might thereby potentially lead to higher fentanyl plasma concentrations. Patients underwent two sample periods and in one period they used aprepitant in a dose schedule which is regularly used to prevent chemotherapy induced nausea and vomiting. Concomitant use of aprepitant showed no statistically significant influence on the AUC of fentanyl in patients with cancer using transdermal fentanyl.

The second study described in this chapter investigated the influence of the localization of the fentanyl patch (thorax versus upper arm) on the exposure of fentanyl. Fentanyl is highly lipophilic and the absorption of transdermal fentanyl might be influenced by skin thickness and/or the amount of subcutaneous fat. We hypothesized that higher plasma concentrations will be reached when the patch is used on areas with thicker skin, as they usually contain more fat. Therefore, we expected differences in fentanyl concentrations between the upper arm and the ventral thorax region for sticking the fentanyl patch, with a lower mean skin thickness

of the thorax compared to the upper arm. PK samples were taken during 2 periods, once after the patch was applied at the upper arm, once when applied to the thorax. Surprisingly, the localization of the patch did not significantly influence fentanyl exposure.

However, in both studies the inter individual variation in plasma fentanyl levels was much larger than we had expected, so both studies were underpowered to find a statistically significant difference.

In **chapter 6** we report a prospective intervention study in patients with head and neck cancer. Painful mucositis and xerostomia (dry mouth) are common problems during and after treatment with curatively aimed chemo- or bioradiation (cisplatin or cetuximab in combination with radiotherapy). We investigated the influence of mucositis and xerostomia on the absorption of sublingually delivered fentanyl, hypothesizing that mucositis may influence plasma levels of fentanyl due to damage of the mucosa. Patients were given a single dose of fentanyl 200 microgram (mcg) sublingually at 4 different time points in their regular treatment schedule. Before start of the therapy, after 3 and 6 weeks (the time points that patients potentially develop severe mucositis) and 6 weeks after radiotherapy (time point with xerostomia). After administration of sublingually delivered fentanyl, fentanyl pharmacokinetic samples were taken. The study showed that mucositis CTC grade ≥ 2 caused by chemo- or bioradiotherapy did not significantly influence the systemic exposure to sublingually delivered fentanyl. Xerostomia led to non-significant lower AUC and fentanyl concentrations compared to baseline. After all, in this clinical setting one of the major problems for patients is having difficulties with swallowing. This study showed that sublingually delivered fentanyl is a safe option for these patients while suffering from grade 2 mucositis .

Discussion

As mentioned before, we were confronted with a higher variation in fentanyl pharmacokinetics than we assumed when making our power analysis for our prospective fentanyl studies. The assumption was a 20% and 25% intra- and inter-patient variability respectively. However, the intra-patient variability we found in the aprepitant, mucositis and the localization studies varied between 28% and 42%, with the lowest variability in the aprepitant study, while the inter- patient variability in the smoking/BMI study was even 87%. Besides the wider variation than expected, the differences in outcomes of the various PK measurements seemed notably smaller than the difference that we had defined as clinically relevant. So, the studied factors are likely to be only minor factors in explaining the variation in fentanyl pharmacokinetics. Consequently, the combination of a wider variation and smaller differences in outcomes make that future studies need significantly more patients to prove an effect of a covariate. However, only covariates that cause a 25% increase of decrease in exposure can be regarded clinically meaningful. Furthermore, some covariates like smoking habits or body size are not suitable to be investigated in a cross-over study.

We performed three of the four studies in patients using a transdermal patch. In one study patients used sublingually delivered fentanyl. Overall, the studies with transdermal patches showed considerably wider variation in fentanyl exposure than the study with sublingual fentanyl, with the exception for the aprepitant study. Probably absorption of transdermal fentanyl is influenced by more divers factors than absorption of sublingual fentanyl. Furthermore blood sampling was performed approximately 24 hours after applying the new fentanyl patch. It is likely that a better defined time schedule of switching the patch and taking blood samples would have led to less variation in the outcomes of fentanyl exposure. Barrett et al found also a 84% variation in the adjusted fentanyl delivery rate in in a large group of 620 patients using transdermal fentanyl while using a single sample design (13). They concluded that multiple clinical factors influenced fentanyl pharmacokinetics but, in line with our findings, they also found that these factors only accounted for a small proportion of the variability.

General conclusions and future perspectives

So, future studies need at least more patients, although it was already challenging to find a sufficient number of eligible and willing to participate patients in our studies. We experienced slow accrual due to a combination of factors: some patients in this fragile population deteriorated rapidly after inclusion and were not able to start or complete the study, other patients did not want to participate because of (extra/ extensive) blood sampling or patients were not asked to participate due to gate keeping of doctors and nurses. Our experiences with pharmacokinetic studies differ from the experiences with early phase clinical studies for which patients are often highly motivated (24). The additional burden associated with the various study procedures without a clear benefit for the participating patient, was a hurdle for a rapid patient inclusion. In future studies patient participation may be promoted by e.g. a limited sampling design (but in combination with strict relation between administration of the drug and sampling times), sampling during routine blood sampling, taking blood samples closer to home or via less invasive sampling techniques, for examples with dried blood spots instead of standard vena punctures (25). Patient participation needs to be increased because of the requirement of larger study populations due to the wide inter- and intra-patient variation. Preferred study

designs are intra patient comparisons due to less variation. Although studies with intravenously administered fentanyl instead of other administration routes would be ideal from research perspective, from clinical perspective future research should also focus on rapid onset opioids (ROO's). The upcoming use of ROO's raises new questions about similarities and differences in pharmacokinetic characteristics of these products in comparison to the transdermal patch in clinical practice.

A second approach to gain more insight in the pharmacokinetics of fentanyl is by combining the various conducted studies and model the pharmacokinetic profile via Population pharmacokinetic modelling using NON-linear Mixed Effects Modelling (NONMEM). This strategy has previously been proven to be useful to explore the optimal switching strategy between continuous subcutaneous fentanyl infusion towards the application of transdermal patches (26). Population pharmacokinetic modelling allows the integration of both poor and rich data and may help to quantify the effect of various covariates and their contribution to fentanyl variability in cancer patients.

Future research might focus on specific groups of patients with cancer e.g. patients with mild to moderate liver failure due to advanced cancer, or patients with multiple comorbidities and co-medication. An example of the influence of comedication is enzalutamide, used to treat patients with *metastasized prostate cancer. Enzalutamide is a inducer of hepatic drug metabolism by induction of the cytochrome CYP 450. A* patient treated with enzalutamide and fentanyl showed increased pain caused by bone metastasis despite fast escalation of the fentanyl dose probably due to the enzalutamide CYP3A4 induction leading to lower plasma levels of fentanyl (27). This observation is confirmed by Benoist et al when they found virtually undetectable concentrations of fentanyl in 6 patients treated with enzalutamide and fentanyl compared to patients treated with abiraterone and fentanyl (28). Another patient population for which pharmacokinetic information is lacking is the group of hospice patients in the last phase of life. These patients still use numerous drugs including all kinds of opioids (29) and pharmacokinetics might be changed by e.g. altered metabolism and distribution. Especially for these fragile patients, knowledge on the indications for adapting the dose of opioids might be helpful. Moreover, future research need to combine pharmacokinetic data and their relationship to clinical effect and side effects to optimize pain treatment.

Although cancer and cancer treatment belong to the hot topics in (clinical) research today, doing research in the field of opioids and cancer seems not to belong to these hot topics. The combination of pharmacokinetic research with old-school opioids in patients with advanced cancer is a combination that did not fit perfectly well in journals focusing on pharmacology, oncology or palliative care, while the topic is interesting for all doctors, nurses, pharmacists etc working in settings with patients with cancer. In the meantime millions of people suffer from cancer and most patients need opioids for cancer related pain somewhere in the trajectory of treatment or the phase of advanced cancer. More attention for this kind of research by doctors, grant givers, policy makers, and scientific journals to publish this kind of work would be very helpful to give this topic the attention that is needed to give the best supportive care to all patients suffering of cancer related pain.

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Appendices

Nederlandse samenvatting

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Samenvatting, discussie en aanbevelingen

Pijn komt veel voor bij patiënten met kanker, vooral bij patiënten met vergevorderde kanker. Om pijn goed te behandelen wordt vaak gebruikt gemaakt van een stappenplan; de WHO pijnladder (pijnladder van de Wereldgezondheidsorganisatie) (1). Stap 3 in deze ladder is het gebruik van sterk werkende opioïden. Opioïden zijn vaak nodig bij de behandeling van matige tot ernstige pijn bij patiënten met kanker. Er zijn veel verschillende soorten opioïden beschikbaar voor de behandeling van pijn, maar in dit proefschrift ligt de nadruk op het geneesmiddel fentanyl. De bekendste vorm van fentanyl is de fentanyl-'pleister' en deze wordt gebruikt voor de behandeling van continu aanwezige pijn. Andere fentanyl-producten worden gebruikt voor de behandeling van doorbraakpijn, zoals tabletten die oplossen in de mond, of een neusspray (2-8).

Voordelen van fentanyl ten opzichte van andere opioïden zijn onder andere de toedieningsmogelijkheid in de vorm van een pleister en het feit dat fentanyl minder obstipatie geeft dan het geneesmiddel morfine. Een groot onderzoek liet zien dat 17% van de patiënten die fentanyl gebruikte rapporteerde last te hebben van obstipatie, terwijl dit in de groep die morfine gebruikte maar liefst bij 48% het geval was (9).

Fentanyl staat bekend om zijn hoge bindingscapaciteit voor plasma-eiwitten en de hoge lipofielheid (5, 10). Lipofiel wil zeggen dat het geneesmiddel door vet aangetrokken wordt of in vet oplosbaar is. Fentanyl wordt omgezet (gemetaboliseerd) in de lever en uitgescheiden door de nieren (11, 12). Deze farmacologische aspecten maken fentanyl geschikt voor opname via de slijmvliezen van mond-neusholte en voor opname via de huid. Een belangrijk nadeel van fentanyl is echter de grote inter- en intra-patiënt variabiliteit in farmacokinetiek (7, 13, 14). Oftewel, dezelfde dosis fentanyl leidt mogelijk niet tot gelijke spiegels van fentanyl in het bloed tussen verschillende patiënten of zelfs binnen dezelfde patiënt.

Factoren die van invloed kunnen zijn op de farmacokinetiek van fentanyl zijn momenteel nog grotendeels onbekend (13, 15-19). Tot nu toe wordt fentanyl, net als andere opioïden, gedoseerd op basis van titratie. Dus de dosis wordt langzaam verhoogd of verlaagd tot een adequaat pijnstillend effect én acceptabele bijwerkingen worden bereikt. Echter, ook tijdens behandeling met een stabiele dosis fentanyl kunnen er periodes zijn waarin de pijn en bijwerkingen opeens erger worden. Soms kunnen deze perioden goed verklaard worden doordat er bijvoorbeeld door het gebruik van een warmtedeken tijdelijk meer fentanyl werd opgenomen uit de pleister dan normaal, of dat er door het gebruik van co-medicatie, welke kan zorgen voor een verlaging van de fentanyl spiegel in het bloed, de pijn minder goed onder controle was (20-23). Over het algemeen is de farmacokinetische variabiliteit echter hoog zonder dat precies duidelijk is welke factoren deze variabiliteit (mede) veroorzaken.

Het primaire doel van dit proefschrift was daarom om de farmacokinetiek van fentanyl bij patiënten met kanker beter te kunnen begrijpen. Daarom hebben we gekeken naar kenmerken van patiënten die aan verandering onderhevig zijn tijdens het ziektebeloop en de behandeling van kanker. We hebben onderzocht of deze patiëntkenmerken van invloed waren op de farmacokinetiek van fentanyl. Als we weten welke factoren van invloed zijn op de farmacokinetiek van fentanyl en deze factoren op tijd herkennen, kan voortijdig de dosis van fentanyl worden aangepast. Vooral in periode van het leven waarin het draait om kwaliteit van leven is een optimale balans tussen het effect en de bijwerkingen van een medicijn uitermate belangrijk.

In dit proefschrift zijn we begonnen met het bestuderen van de literatuur om relevante factoren te vinden die bestudeerd werden met betrekking tot de farmacokinetiek van fentanyl. Daarna hebben we een aantal prospectieve studies uitgevoerd om gemeenschappelijke klinische variabelen van de patiënt te bestuderen die gevoelig zijn voor verandering tijdens het ziekteproces in patiënten met kanker en die mogelijk de fentanyl-farmacokinetiek zouden kunnen beïnvloeden.

Hoofdstuk 2 beschrijft de resultaten van een systematisch review van de factoren die onderzocht zijn en die mogelijk de farmacokinetiek van fentanyl beïnvloeden. Met een systematische Pubmed-, Cochrane- en Embase-zoekopdracht identificeerden we 31 onderzoeken die voldeden aan onze inclusiecriteria om farmacokinetische parameters te beschrijven zoals klaring, 'Area Under the Curve' (AUC) en tijd tot maximale concentratie (T_{max}). In totaal werden 36 factoren gevonden en onderverdeeld in 4 groepen: interacties met geneesmiddelen, omgevingsfactoren (bijv. het verwarmen van de pleister of de plek waar de pleister geplakt is), patiëntgerelateerde factoren (bijv. leeftijd en body mass index (BMI)) en tenslotte genetische variaties. We zagen een enorme variabiliteit in de gevonden studies. Het ging bijvoorbeeld in sommige gevallen om onderzoek in vrijwilligers in plaats van patiënten, er waren hele kleine studies versus grotere studies, er was verschil in hoe bloedafnames voor farmacokinetiek plaatsvonden en verder werden de snel werkende fentanyl-producten slechts in een klein aantal studies onderzocht. Er waren helaas geen studies die de farmacokinetiek bestudeerden in relatie tot de klinische effecten. We zagen dat gebruik van zogenaamde CYP3A4-remmers en -inductoren (bepaalde soorten medicijnen die op dit levereiwit inwerken), verminderde leverfunctie en opwarming van de pleister daadwerkelijk de blootstelling aan fentanyl konden beïnvloeden. De invloed van andere factoren was minder duidelijk. Bij oudere patiënten suggereerden de gegevens dat een zorgvuldige dosering van fentanyl vanwege veranderingen in absorptie en metabolisme aan te raden is. De invloed van BMI en geslacht op de farmacokinetiek van fentanyl was twijfelachtig; hoogstwaarschijnlijk vanwege een grote heterogeniteit in de gepubliceerde studies. Wanneer we kijken naar variatie in het DNA van eiwitten die bij fentanyl afbraak betrokken zijn (farmacogenetica), zoals bijvoorbeeld het *CYP3A5*3*-genpolymorfisme, dan is verder

onderzoek zeer gewenst om daadwerkelijk te kunnen bepalen of dit van invloed zou kunnen zijn op fentanyl-farmacokinetiek. Onze aanbevelingen voor toekomstig onderzoek waren om te focussen op patiënten in verschillende fases van ziekte, om onderzoek te doen bij het gebruik van verschillende fentanyl-producten en om te kijken naar de relaties tussen farmacokinetiek en effect (zowel pijn als bijwerkingen). Deze aanbevelingen waren ons startpunt voor de onderzoeken die we daarna hebben uitgevoerd.

Hoofdstuk 3 beschrijft de validatie van een methode om diverse opioïden te kunnen meten in plasma. Het gaat hierbij om de opioïden morfine, fentanyl, hydromorfon en de gevormde metabolieten norfentanyl, morfine-3ß-glucuronide en morfine-6ß-glucuronide.

Met een ondergrens van kwantificering voor fentanyl van 0.1 ng /ml was deze methode zeer geschikt (en noodzakelijk) om de uitvoering van de farmacokinetische onderzoeken met fentanyl beschreven in dit proefschrift te ondersteunen.

In de studie beschreven in **hoofdstuk 4** hebben we in patiënten met kanker die een fentanylpleister gebruikten, het effect van roken en BMI op de farmacokinetiek van fentanyl prospectief onderzocht. Het ging om een verkennend cohortonderzoek waarbij gebruikt gemaakt werd van de afname van bloedmonsters voor farmacokinetisch onderzoek. Er werd eenmalig een bloedmonster afgenomen, ongeveer 24 uur na het vervangen van een nieuwe fentanylpleister op de bovenarm. In totaal waren 88 patiënten evalueerbaar voor dit onderzoek. Er werden geen statistische verschillen gevonden in plasma-fentanylconcentraties voor de groep rokers in vergelijking met niet-rokers, hoewel onze gegevens een lagere blootstelling aan fentanyl in de rokende groep suggereerden. We vonden ook geen statistische verschillen voor de groep patiënten met een lage BMI (<20 kg/m^2) in vergelijking met de groep patiënten met een hoge BMI ($>$ 25 kg/m^2). Op basis van dit onderzoek hebben patiënten vooraf geen dosisaanpassing nodig vanwege hun BMI of het feit of ze wel of niet roken. Een belangrijke bevinding in ons onderzoek was dat er een grotere inter-patiënt variatie werd gevonden dan we van tevoren hadden verwacht op basis van eerdere fentanylstudies. Hoewel de exacte verklaring voor deze variatie onduidelijk is, kan dit wel de uitkomst van onze studie hebben beïnvloed. Voor toekomstige studies in deze patiëntenpopulatie moet men dus rekening houden met deze grote variabiliteit. Wellicht is deze variatie hoger in onze onderzochte populatie dan in studies met andere patiënten of gezonde vrijwilligers.

In **hoofdstuk 5** beschrijven we de resultaten van twee prospectieve farmacokinetische interventiestudies. In beide onderzoeken ging het om 14 evalueerbare patiënten, werden intra-patiënt vergelijkingen gemaakt en werden deze uitgevoerd bij patiënten met kanker die een fentanylpleister gebruikten om hun pijn te behandelen. De eerste

studie onderzocht de invloed van het gebruik van het geneesmiddel aprepitant op de blootstelling aan fentanyl. Aprepitant is een matige CYP3A4-remmer en een veelgebruikt medicijn voor de behandeling en preventie van misselijkheid en braken veroorzaakt door chemotherapie. De hypothese was dat aprepitant een remmend effect op CYP3A4 kon hebben en daardoor tot een hogere fentanylconcentratie leidde. Er waren twee periodes waarin patiënten bloedafnames ondergingen, en in één van deze periodes gebruikten patiënten aprepitant (volgens het zogenaamde cross-over design). De aprepitant werd gedoseerd volgens het reguliere schema als voorgeschreven in de dagelijkse praktijk voor het voorkomen van misselijkheid en braken tijdens chemotherapie. Gelijktijdig gebruik van aprepitant en fentanyl liet echter geen statistisch significante invloed zien op de AUC van fentanyl, waardoor deze middelen veilig te combineren zijn.

De tweede studie beschreven in dit hoofdstuk onderzocht de invloed van de plaats waar de fentanylpleister werd geplakt (borstkas of bovenarm) op de blootstelling van fentanyl. Fentanyl is zoals genoemd lipofiel en de absorptie van transdermaal fentanyl kan worden beïnvloed door de dikte van de huid en/of de hoeveelheid onderhuids vet. De hypothese achter deze studie was dat er een hogere plasmaconcentratie kon worden bereikt wanneer de pleister werd gebruikt op plaatsen met een dikkere (en dus meer vet bevattende) huid. Doordat de gemiddelde huiddikte op de borstkas lager is dan op de bovenarm verwachtten we een lagere spiegel van fentanyl wanneer patiënten de pleister op de borstkas plakten in vergelijking met het plakken van de pleister op de bovenarm. Bloedafnames ten behoeve van farmacokinetiek bepalingen werden verricht gedurende 2 periodes, in één periode werd de pleister op de borstkas geplakt, in de andere periode op de bovenarm. Uiteindelijk bleek de plek van de pleister de blootstelling aan fentanyl echter niet significant te beïnvloeden, dus patiënten kunnen zelf kiezen op welk van de twee plekken ze de pleister het liefste plakken.

In **hoofdstuk 6** beschrijven we een prospectieve interventiestudie bij patiënten met hoofd- en halskanker. Mucositis (slijmvliesontsteking van mond/keel) en xerostomie (droge mond) zijn veel voorkomende problemen tijdens en na behandeling met op genezing gerichte chemo- of bioradiotherapie (dat wil zeggen: het geneesmiddel cisplatin of cetuximab in combinatie met radiotherapie). We onderzochten de invloed van mucositis en xerostomie op de opname van fentanyl bij toediening in een tablet onder de tong (sublinguaal), waarbij de hypothese was dat mucositis de plasma-spiegels van fentanyl kan beïnvloeden als gevolg van beschadiging van het slijmvlies. Patiënten kregen op 4 verschillende tijdstippen in hun reguliere behandelingsschema een tablet van 200 microgram fentanyl onder de tong. De eerste gift was voor aanvang van de behandeling, daarna na 3 en 6 weken (het tijdstip waarop patiënten potentieel ernstige mucositis ontwikkelen) en tot slot 6 weken na radiotherapie

(tijdstip van xerostomie). Na toediening van fentanyl werden bloedafnames verricht ten behoeve van farmacokinetische bepalingen. De studie toonde aan dat mucositis graad 2 of hoger (volgens een veelgebruikte classificatie methode), veroorzaakt door chemo- of bioradiotherapie, de blootstelling aan sublinguaal fentanyl niet significant beïnvloedde. Xerostomie zorgde voor een niet-significante lagere AUC en fentanyl-concentraties in vergelijking met de situatie voor start van de behandeling. Belangrijk te realiseren is dat patiënten in deze behandeling een groot probleem hebben met het slikken van medicatie. Deze studie laat zien dat sublinguaal fentanyl een goede optie kan zijn in deze categorie patiënten.

Zoals eerder al genoemd werden we geconfronteerd met een veel grotere variatie in de farmacokinetiek van fentanyl dan we tevoren hadden aangenomen bij het berekenen van onze poweranalyse voor de prospectieve fentanylstudies. Deze aannames waren gebaseerd op respectievelijk 20% en 25% intra- en interpatient variabiliteit. De intra-patiënt variabiliteit die we echter vonden in de aprepitant-, mucositis- en pleisterplaats studies varieerde tussen 28% en 42%, , terwijl de inter-patiënt variabiliteit in de rokers-/BMI-studie zelfs 87% was. Naast de grotere spreiding dan verwacht, leken ook de gevonden verschillen in uitkomsten van de verschillende studies kleiner dan het verschil dat we als klinisch relevant hadden gedefinieerd. De bestudeerde factoren zijn dus waarschijnlijk slechts relatief kleine factoren bij het verklaren van de variatie in de farmacokinetiek van fentanyl. De combinatie van deze grotere spreiding en kleine verschillen in uitkomsten maakt daarom dat toekomstige studies waarschijnlijk significant meer patiënten nodig hebben om een effect van een co-variabele factor te bewijzen. Cross-over onderzoek verdient daarbij de voorkeur; echter sommige co-variabelen zoals het wel of niet roken en de hoogte van het BMI zijn niet geschikt om in een cross-over onderzoek te onderzoeken.

Drie van de vier beschreven onderzoeken werden uitgevoerd bij patiënten die een fentanylpleister gebruikten. In één studie werd gebruik gemaakt van sublinguaal fentanyl. Over het geheel genomen vertoonden de onderzoeken met fentanylpleisters een aanzienlijk grotere variatie in blootstelling aan fentanyl dan het onderzoek met sublinguaal fentanyl, met uitzondering van de aprepitant-studie. Het lijkt waarschijnlijk dat de absorptie van fentanyl uit de pleister door meer factoren wordt beïnvloed dan absorptie van sublinguaal fentanyl. Daarnaast werden bloedafnames ongeveer 24 uur na het plakken van de nieuwe pleister uitgevoerd; mogelijk had een striktere planning van de wissel van de pleister en het tijdstip van de bloedafname tot minder variatie geleid. Ook in eerder beschreven onderzoek werd een grote inter-patiënt variatie (84%) gevonden. Deze onderzoekers keken hierbij naar de snelheid van fentanyl afgifte in een grote groep patiënten en ook hier werd eenmalig een bloed-

afname gedaan voor analyse van fentanyl farmacokinetiek (13). Zij concludeerden dat de farmacokinetiek van fentanyl door meerdere klinische factoren tegelijk werd beïnvloed, maar, in overeenstemming met onze bevindingen, was dit slechts een verklaring voor een klein deel van de variabiliteit.

Het bleek een grote uitdaging om voldoende bereidwillige patiënten te vinden voor deelname aan deze studies. De inclusie in de studies verliep moeizaam door een combinatie van factoren. Sommige patiënten in deze kwetsbare populatie verslechterden snel na inclusie en konden het onderzoek niet starten of voltooien, andere patiënten wilden niet deelnemen vanwege (extra / uitgebreide) bloedafname of patiënten werd niet gevraagd om deel te nemen vanwege het 'gate-keeping-effect' door artsen en verpleegkundigen. De moeizame inclusie in dit type farmacokinetisch onderzoek verschilt met de ervaringen met vroege fase klinische studies waar inclusie vaak soepeler verloopt en patiënten zeer gemotiveerd zijn om deel te nemen (24). Mogelijk was de extra belasting van het onderzoek, zonder hoop op een duidelijk voordeel voor de deelnemende patiënt van invloed op de trage inclusie. In toekomstige studies kan wellicht inclusie van patiënten worden bevorderd door te streven naar minimale bloedafnames (maar wel in combinatie met een strikte relatie tussen toediening van het geneesmiddel en tijden van de bloedafnames), bloedafnames tijdens standaard bloedafnames, bloedafnames dichterbij huis of via minder invasieve technieken, zoals bijvoorbeeld met 'dried-blod-spots' (25).

Hoewel studies met intraveneus toegediende fentanyl in plaats van andere toedieningsroutes vanuit onderzoeksperspectief ideaal zouden zijn, zou toekomstig onderzoek, vanuit klinisch perspectief geredeneerd, zich ook meer moeten focussen op snel de snel werkende opioïden (ROO's). Het steeds veelvuldiger gebruik van ROO's roept nieuwe vragen op over overeenkomsten en verschillen van de farmacokinetische eigenschappen van de ROO's ten opzichte van de pleister.

Een tweede benadering om meer inzicht te krijgen in de farmacokinetiek van fentanyl is door de verschillende uitgevoerde onderzoeken te combineren en het farmacokinetische profiel te modelleren via farmacokinetische populatie modellen. Het is eerder bewezen dat deze benadering zinvol is, kijkend naar hoe om te gaan met doseren van fentanyl bij het wisselen tussen subcutane fentanyl toediening en overstappen naar de fentanylpleister (26). Farmacokinetische populatie modellering maakt het mogelijk om zowel beperkte als uitgebreide gegevens-sets te integreren om daarmee beter te kunnen beoordelen welke verschillende co-variaten bijdragen aan de variabiliteit in de farmacokinetiek van fentanyl bij kankerpatiënten en hoe groot die betreffende bijdragen zijn.

Toekomstig onderzoek zou zich ook moeten richten op specifieke groepen binnen de gehele populatie patiënten met kanker. Bijvoorbeeld patiënten met mild tot matig leverfalen als gevolg van uitgezaaide kanker, of patiënten met comorbiditeit en co-medicatie. Een voorbeeld van de invloed van co-medicatie is enzalutamide; dit wordt gebruikt voor de behandeling van patiënten met gemetastaseerde prostaatkanker. Enzalutamide zorgt voor inductie van CYP3A4 eiwit in de lever. Een beschrijving van een patiënten casus liet zien dat een patiënt behandeld met tegelijkertijd enzalutamide en fentanyl, toename van pijn ervaarde bij bekende botmetastasen, ondanks het (snel) ophogen van de fentanyl. Waarschijnlijk zorgde de combinatie van enzalutamide en fentanyl, door inductie van het CYP voor een lagere spiegel van fentanyl, met meer pijn tot gevolg (27). Deze observatie werd bevestigd door anderen toen ze vrijwel niet-detecteerbare fentanyl-concentraties vonden bij een groep patiënten behandeld met enzalutamide en fentanyl, vergeleken met patiënten behandeld met abiraterone en fentanyl (28). Abiraterone is ook een geneesmiddel voor de behandeling van prostaatkanker, maar beïnvloedt het CYP niet zoals enzalutamide dat wel doet.

Een andere patiëntenpopulatie waar farmacokinetische informatie praktisch ontbreekt, is de groep hospice-patiënten in de laatste levensfase. Deze patiënten gebruiken nog steeds talloze geneesmiddelen waaronder allerlei opioïden (29) en de farmacokinetiek in deze fase zou beïnvloed kunnen worden door het veranderde metabolisme en verandering in de verdeling (distributie) van geneesmiddelen door het lichaam. Vooral voor deze kwetsbare groep patiënten kan kennis over de indicaties voor het aanpassen van de dosering van de opioïden nuttig zijn.

Tot slot moet toekomstig onderzoek vooral data van farmacokinetisch onderzoek combineren met het effect op klinische eindpunten om zo de pijnbehandeling te kunnen optimaliseren.

Hoewel kanker en kankerbehandeling absoluut behoren tot de meest besproken onderwerpen in de onderzoeksagenda van tegenwoordig, behoort momenteel het onderzoek naar opioïden zeker niet tot het meest besproken onderzoek. In de tussentijd hebben miljoenen mensen kanker-gerelateerde pijn en krijgen zij ergens in hun ziekte en of behandelingstraject opioïden voorgeschreven. Meer aandacht voor dit soort onderzoek door zowel dokters, subsidie/beurs verstrekkers, beleidsmakers en wetenschappelijke tijdschriften kan zeer behulpzaam zijn om dit onderwerp zichtbaarder te maken. Dit is cruciaal om uiteindelijk de best mogelijke ondersteunende zorg te kunnen geven aan alle patiënten de lijden aan pijn veroorzaakt door kanker.

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Curriculum Vitae

Evelien Kuip werd op 17 november 1979 geboren in Groenlo. In 1998 behaalde zij haar VWO diploma aan het R.K.S.G. Marianum in Groenlo. Zij studeerde geneeskunde aan de Radboud universiteit in Nijmegen. In 2005 haalde ze haar artsexamen. Daarna werkte ze als ANIOS en later als AIOS interne geneeskunde in het Slingeland Ziekenhuis in Doetinchem (opleider dr. A.H. Mudde). In 2009 werd de opleiding vervolgd in het Radboud Ziekenhuis in Nijmegen (opleider prof.dr. J. de Graaf). In 2010 startte zij met de opleiding binnen het aandachtsgebied oncologie in het Erasmus MC in Rotterdam (opleider dr. A. van der Gaast). Van 2012 tot 2015 werkte ze als chef de clinique op de afdeling medische oncologie en combineerde dit met het doen van onderzoek, beschreven in dit proefschrift. Vanaf 2015 werkt zij als staflid op de afdeling Interne Oncologie (afdelingshoofd prof.dr.ir. J.J.M. Van der Hoeven) en de afdeling Anesthesiologie, Pijn en Palliatieve Geneeskunde in het Radboud UMC (afdelingshoofd prof.dr. G.J. Scheffer). Het proefschrift is tot stand gekomen onder supervisie van prof.dr. A.H.J. Mathijssen, prof.dr. C.C.D. Van der Rijt en dr. S.L.W. Koolen.

Publications

Kuip EJM, Muller E. Fatal pneumonitis after treatment with docetaxel and trastuzumab. *Neth. J. Med. 2009; 67(6):237-239.*

Kuip EJM, Zandvliet ML, Mathijssen RHJ, Van der Rijt CCD. Pharmacological and clinical aspect of immediate release fentanyl preparations: criteria for selection. *Eur J Hosp Pharm 2012;19:38-40.*

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de Bruijn P, **Kuip EJM**, Lam MH, Mathijssen RHJ, Koolen SLW. Bioanalytical methods for the quantification of hydromorphone, fentanyl, norfentanyl, morphine, morphine-3ß-glucuronide and morphine-6ß-glucuronide in human plasma. *J. Pharm. Biomed. Anal. 2018;149:475-481.*

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Kuip EJM, Oldenmenger WH, Visser-Thijs MF, de Bruijn P, Oomen-de Hoop E, Mathijssen RHJ, van der Rijt CCD, Koolen SW. Influence of aprepitant and localization of the patch on fentanyl exposure in patients with cancer using transdermal fentanyl. *Oncotarget 2018;9(26):18269-18276.*

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Kuip EJM, Oldenmenger WH, Oomen-de Hoop E, Verduijn G, Thijs-Visser MF, De Bruijn P, Van Meerten E, Koolen SLW, Mathijssen RHJ, Van der Rijt CCD. Pharmacokinetics of sublingually delivered fentanyl in head and neck cancer patients treated with curatively aimed chemo- or bioradiotherapy. *Cancers 10(11): 445, 2018.*

PhD Portfolio

Summary of PhD training and teaching

Name PhD student:	PhD period: January 2012 – November 2018
Evelien Kuip	Promotor(s): Prof.dr. A.H.J. Mathijssen,
Erasmus MC Department:	Prof.dr. C.C.D. van der Rijt
Medical Oncology	Copromotor: Dr. S.L.W. Koolen

1. PhD training

	Year	Workload (Hours/ ECTS)
General academic skills		
Research Integrity		
Good clinical practice	2012	0.5
Statistics; introduction SPSS	2012	0.5
Methodology (Methodologie van patiëntgebonden onderzoek en voorbereiding van subsidie-aanvragen)	2012	0.25
Biomedical English Writing and Communication	2013	1.5
BROK (‘Basiscursus Regelgeving Klinisch Onderzoek’	2013	1
BROK recertification	2017	0.5
In-depth courses		
8-day Course Palliative care for medical specialists	2015	2.5
Presentations		
Oral presentation IKNL Rotterdam	2012	0.5
Oral presentation Research meeting Public Health and End of Life care	2012	0.5
Poster presentation EAPC Trondheim	2012	1
Oral presentation National Congress Palliative Care Lunteren	2012	1
Poster presentation Therapie op Maat	2012	1
Oral presentation Palliative care symposium Erasmus MC 2012:	2012	1
Oral presentation EAPC Amsterdam	2014	1
Oral presentation Research Meeting Erasmus MC Medical Oncology	2016	1
Oral presentation ‘Jonge oncologen Avond’	2017	1
Web lecture ‘Nieuwe richtlijn pijn bij kanker’	2017	1
Poster presentation EAPC Oslo	2017	1
Oral presentations (immunotherapy/palliative care; International symposium Nottwill, Switzerland	2018	2

Seminars and workshops

OMBO courses department of medical oncology	2012-2014	0.5
Research meeting Erasmus MC	2012-2014	0.5
Regional Pain clinic (region Randstad/Nijmegen)	2013, 2014, 2017,2018	4
Workshop communicating with cancer patients	2013	0.3
OMBO courses department of medical oncology	2012-2014	0.5

(Inter)national conferences

Post-EAPC	2013-2017	0.5
ESMO 2013/2014/2018	2013-2014	3.6
Year symposium 'continuum oncology'	2014	0.3
NVMO congress	2013-2018	2.2
'IKNL netwerkdagen'	2013	1.2
'De dokter en de dood'	2015	0.5
San Antonio 'in het bos'	2015-2017	3.6
ASCO	2016	1.2
Masterclass Malignant Bowel Obstruction by Peritonitis Carcinomatosa	2017	1.2
Evidence based supportive oncology	2017	1.2
NABON/BOOG symposium	2018	0.5
EAPC Bern	2017	1.2

Other

Consultation meetings palliative care teams region Nijmegen	2017-2018 2016-2018	1.5 1
IKNL meetings Breast cancer		

2. Teaching

	Year	Workload (Hours/ ECTS)
Didactic skills		
Education AIOS/ANIOS Erasmus MC	2012-2014	3
Education AIOS Radboud UMC	2015-2018	3
Education nurse practitioners Radboud UMC	2015-2018	2
Start qualification teaching	2017	0.5
Supervising research projects students medicine and biomedical sciences	2018	2

Lecturing

OIO meetings 'pharmacokinetics of fentanyl'	2013-2017	2
Oncology nurse teaching days	2013-2014	2
Research meeting Internal Medicine	2016	1
Breast cancer	2015-2018	1
Minor breast cancer and research	2017	0.5
Basic curriculum Palliative Care	2017-2018	1
Minor Palliative Care	2018	1
Teaching sessions palliative care	2018	1